California Energy Commission
Alternative and Renewable Fuel and Vehicle Technology Program

FINAL PROJECT REPORT

Energy-Efficient and Cost-Effective Microalgae Disruption for Extraction of Lipids for Biodiesel Production

Prepared for: California Energy Commission

Prepared by: San Diego State University Research Foundation

SAN DIEGO STATE UNIVERSITY

California Energy Commission
Gavin Newsom, Governor

April 2019 | CEC-600-2019-015

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Contract Number: ARV-15-011

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PREFACE

Assembly Bill (AB) 118 (Nùñez, Chapter 750, Statutes of 2007), created the Alternative and Renewable Fuel and Vehicle Technology Program (ARFVTP). The statute authorizes the California Energy Commission (Energy Commission) to develop and deploy alternative and renewable fuels and advanced transportation technologies to help attain the state's climate change policies. AB 8 (Perea, Chapter 401, Statutes of 2013) re-authorizes the ARFVTP through January 1, 2024, and specifies that the Energy Commission allocate up to $20 million per year (or up to 20 percent of each fiscal year's funds) in funding for hydrogen station development until at least 100 stations are operational.

The ARFVTP has an annual budget of approximately $100 million and provides financial support for projects that:

- Reduce California's use and dependence on petroleum transportation fuels and increase the use of alternative and renewable fuels and advanced vehicle technologies.
- Produce sustainable alternative and renewable low-carbon fuels in California.
- Expand alternative fueling infrastructure and fueling stations.
- Improve the efficiency, performance and market viability of alternative light-, medium-, and heavy-duty vehicle technologies.
- Retrofit medium- and heavy-duty on-road and non-road vehicle fleets to alternative technologies or fuel use.
- Expand the alternative fueling infrastructure available to existing fleets, public transit, and transportation corridors.
- Establish workforce training programs and conduct public outreach on the benefits of alternative transportation fuels and vehicle technologies.

To be eligible for funding under the ARFVTP, a project must be consistent with the Energy Commission's ARFVTP Investment Plan, updated annually. The Energy Commission issued PON-14-602 to fund projects that put emphasis on transformative technology solutions to significant biofuels industry problems that increase yields, productivity, or cost effectiveness of biofuel production; and/or that target a significant unmet need in California's biofuels industry. In response to PON-14-602, the recipient submitted an application which was proposed for funding in the Energy Commission's Notice of Proposed Awards June 24, 2015 and the agreement was executed as ARV-15-011 on December 28, 2015.

Please use the following citation for this report:

Garoma, Temesgen. San Diego State University. 2019. *Energy-Efficient and Cost-Effective Microalgae Disruption for Extraction of Lipids for Biodiesel Production.* California Energy Commission. Publication Number: CEC-600-2019-015.

ABSTRACT

This project investigated the use of Cutrine plus and copper sulfate for the disruption of algal biomass. The chemicals effectively disrupted algae cells, enhancing lipid extraction. The quantity of lipid extracted increased by about 30% for disrupted samples. Moreover, the estimated energy input, GHG emission, and operating cost for the proposed cell disruption method were lower than those for the existing methods. The estimated energy inputs were 5 to 300 times lower than those for the existing methods, while the GHG emissions were 8 to 600 times lower. On the basis of operating cost, copper sulfate ranked at 30 percentile and Cutrine plus at 60 percentile compared to the existing methods.

Despite these advantages, the adaption of the method may be limited by the extended (about 24-hour) contact time required to achieve significant increase in lipid extraction. Moreover, algal biofuel is not currently competitive with petroleum-based fuels, mainly due to cost and energy intensity. The integration of algal biofuel production with waste treatment systems was recommended to address this challenge. The integration could allow for the recovery and unitization of resources contained in various waste streams.

If these challenges are addressed with future research efforts, then algal biofuel can provide several potential benefits: a) it has an estimated GHG emissions of 55.25 gCO_2-e/MJ of biodiesel produced, which is below the California Health & Safety requirement of 83.25 gCO_2-eq/MJ for diesel substitute. b) it has lower water consumption intensity than the majority of feedstocks used or considered for biofuel production. c) the industry could create about 72,000 jobs in California. d) it could enhance natural resources preservation by recovering and utilizing resources contained in various waste streams.

Keyword*s*: *C. vulgaris,* cell disruption, lipid extraction yield, Cu, copper sulfate, GHG emission, water usage, and resource recovery and utilization.

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

1. Executive Summary

Microalgae have emerged as a promising long-term and sustainable feedstock for biofuel production due to their high productivity rate, ability to tolerate a wide range of growth conditions, and lack of competition for land and water with food crops. A considerable amount of public and private funding has been spent on algal biofuel research, development, and demonstration. The basic concept of using algal biomass as feedstock for biofuel production has been proven and demonstrated. However, a scalable and commercially viable system has yet to be developed. The key barriers relate to the cost and energy intensity of the algae-to-biofuel pathway process.

The main steps in the pathway include 1) algae culture cultivation, 2) algal biomass harvesting/dewatering, 3) algal biomass disruption or pre-treatment, 4) algal lipid extraction, 5) algal lipid conversion, and 6) algal biofuel.

Cu and copper sulfate were used in this project and were shown to be effective in disrupting algae cells. Sample results confirmed that the algae cells were ruptured after treatment with these agents.

The benefits of using Cu and copper sulfate as algal biomass disruption methods were evaluated on the basis of energy input, GHG emission, and operating cost. With respect to Cu and copper sulfate, the estimated specific energy requirements were 5 to 300 times lower than the requirements for existing algae cell disruption methods, and the GHG emissions were 8 to 600 times lower than those for existing methods. With respect to operating costs, it was determined that Cu and copper sulfate ranked lower when compared to existing algae cell disruption methods.

Despite these results, algal biofuel is not currently competitive with petroleum-based fuels, mainly related cost and energy intensity. The cost of algal-biofuel production varied from $3.00 to $29.8 per gallon for biodiesel and from $12.80 to $153.40 per gallon for a precursor feedstock for biodiesel production. About 60% to 75% of the overall cost was attributed to capital costs, while 25% to 40% to operating costs.

While the project results point out that algal biofuel production is not competitive with fossil technology, it also appears that algal solutions for the transportation sector requires significant investments, technological development, and time. It is not clear that the private sector is willing to make the requisite investments needed or that the public sector has the capacity to invest in this technology for the long term in reducing technological costs. Equally evident is the absence of when the public may see commercial algal products in the transportation sector.

It is recommended that the technology must be pilot-tested to identify and address challenges that may arise during upscaling. The data that will be gathered from pilot-scale tests would be used to establish the process and operational parameters for the technology, paving the way for eventual commercialization.

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2. CHAPTER 1: Introduction

2.1. Background

Senate Bill (SB) 32 was approved by Governor Brown in 2016. SB 32 requires the California Air Resources Board (CARB) to ensure that statewide GHG emissions are reduced 40% below 1990 levels by 2030.

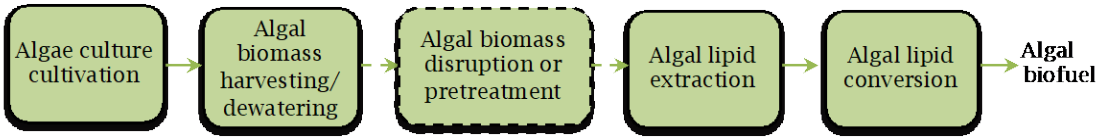
The 2016 CARBs statewide GHG emission inventory indicates that the transportation sector is the largest source of GHG in California, responsible for 50% of emissions when fuel refining is included, as well as 80% of smog-forming pollutants.

Executive Order B-55-18 indicates that while California has taken specific steps to reduce GHG emissions, Governor has taken further steps to reduce climate change impacts. His executive order established a new statewide goal "to achieve carbon neutrality as soon as possible, no later than 2045, and achieve and maintain net negative emissions thereafter."

Microalgae have emerged as a promising long-term and sustainable feedstock for biofuel production due to their high productivity rate [5], ability to tolerate a wide range of growth conditions [6], and lack of competition for land and water with food crops [7]. Moreover, CO_2 sequestration via algae was estimated to be one to two orders of magnitude greater than terrestrial plants [8]. A considerable amount of funding, from both government and private investments, has been spent on research, development, and demonstration projects focused on algal biofuel. As a result, the basic concept of using algal biomass as feedstock for biofuel production has been proven and demonstrated [9]. However, a scalable, sustainable, and commercially viable system has yet to be developed. The key barriers relate to the cost and energy intensity of the processes involved in the algae-to-biofuel pathway.

Currently, two approaches are pursued in the algae-to-fuel pathway: (1) algal lipid extraction and upgrading, and (2) whole algae hydrothermal liquefaction and upgrading. In the former pathway, microalgae cultivation, harvesting/dewatering, extraction, and conversion are the main steps involved (Figure 1). Of these steps, the processes used for algae cultivation and the conversion of extracted cell contents to biofuel are relatively well-established. Algal biomass harvesting and extraction of lipids still attract intense interest from researchers around the world [10].

Figure 1: Main steps in algal lipid extraction and upgrading pathway



One of the main hurdles in the extraction step is the recalcitrant nature of the algae cell walls due to the presence of complex biopolymers such as microfibrillar polysaccharides, matrix polysaccharides and proteoglycans [11]. To overcome this, algal biomass disruption or pretreatment prior to the extraction step was proposed and being investigated by various researchers to enhance the lipid recovery. In turn, the current disruption methods have their own limitations. A majority of the them are adapted from the food industry, where energy-efficiency and cost-effectiveness are less of a factor of viability for a technology since food products can command a high price on the market.

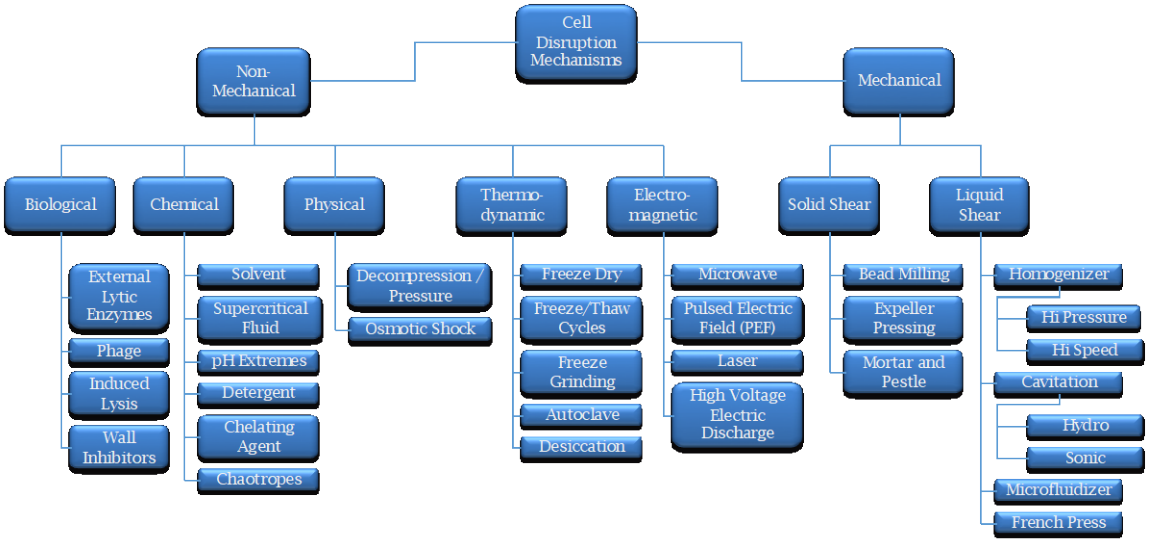
The focus of this project was on developing new methods for algal biomass disruption or pretreatment.

2.2. The Current State of Algal Biomass Disruption Technologies

Improving the efficiency of algal biomass pretreatment and bio products extraction is a concrete and independent way to improve outcomes of TEA and energy return on investment (net energy) for algae biofuel. Because the majority of desirable cell materials lie within the cell, efficient recovery requires cell rupture [12]. One way to improve overall net energy is to maximize the efficiency of cell rupture during algal biomass pretreatment.

The disruption and disintegration of microbial biomass is a relatively mature field. Summaries of modern classifications of cell disruption methods appear as early as 1971 [13] and likely extend further back than that. While the design objective is a mechanical failure of the cell wall and membrane, this objective can be accomplished with mechanical and/or non-mechanical mechanisms. The taxonomy of cell disruption technologies (Figure 2) begins with this distinction between mechanical and non-mechanical methods.

Figure 2: Existing algal cell disruption methods



Source: adapted and modified from [12-18].

Mechanical methods of cell disruption are characterized by the direct application of force as a surface force. These surface forces are applied in the forms of solid and liquid shear. Three methods that fall into the solid shear category are bead milling, expeller pressing, and grinding with a mortar and pestle. In bead milling, a solution containing microbial biomass is fed into a chamber partially filled with inert beads. The beads in the chamber are then agitated either by shaking or by rotors inside the chamber. These moving beads directly impact and crush the cells in solution. An expeller press is a combination of the principles of an Archimedes screw, a centrifuge, and a screen. Solution containing biomass is fed into a tubular chamber tightly fitted with a screw conveyor and wrapped with a fine screen. The screw is rotated at high speed, creating a high-pressure environment with large centrifugal force. The heavier solid materials accumulate against the screen, and liquids are harvested either through the center of the screw (usually in dewatering applications) or as they pass through the screen (usually in oil recovery applications). The solid shear in this case is imparted by the screw, the screen, and the impingement of the cells on one another. Grinding with a mortar and pestle involves the manual grinding of solids between two solid surfaces by pressing the surfaces together longitudinally and then moving them transversely.

Liquid shear methods are grouped here by the creation of highly localized pressure and velocity gradients within a fluid. These can be present alone, or they can be combined with a solid surface on which materials in the fluid impinge. Four groups of processes in this classification are homogenization, cavitation, micro fluidization, and French press.

Non-mechanical methods of cell disruption are more diverse, being characterized by the absence of direct application of force as a surface force. These can be identified as the modification of the cells' environment to impart new, or take advantage of existing, body forces. These body forces then translate to surface forces in the context of a closed surface in local tension or compression, namely the cell. These non-mechanical methods can be categorized as: electromagnetic, thermodynamic, pressure, chemical, and biological.

Cell disruption methods and microalgae combinations are nearly as diverse as the number of papers in the literature on the subject. Three of the most prevalent cell disruption methods are sonication, freeze drying, and grinding. However, freeze dry and grinding skew the data a bit as these two methods are the standard sample preparation steps for extraction of lipids by supercritical fluid. The next most prevalent methods are microwave and thermal, followed by high pressure homogenization, autoclaving, and bead beating.

Table 1 summarizes (adapted from Lee et al. 2012 [16]) the specific energy use during different disruption experiments performed by various researchers. The energy use quoted in each publication has been converted to specific energy use, with units of MJ per kg of dry mass, which allows the energy consumption by each method to be compared. The results show that hydrodynamic cavitation has the lowest energy

requirement at 33 MJ per kg of dry algal cells [16]. This figure doesn't include energy inputs for the cultivation, harvesting/dewatering, extraction, and conversion to biofuels. The energy available by the combustion of the entire algal biomass was estimated to be about 29 MJ per kg of dry cells [16]. Therefore, the existing cell disruption methods result in a negative net energy balance.

Table 1: Summary of experimental cell disruption methods and their energy consumptions

Methods	Material and experimental conditions (disruption volume, concentration, power consumption, disruption duration)	Calculated energy use (GJ/m^3 cell suspension)	Energy use MJ/ kg dry mass	Scale of use
Sonication	*Chlorococcum sp* (0.2 L, 8.5 kg/m^3, 750 W, 5 min)	1.12	132	Laboratory, industrial
High Pressure Homogenizer	*Chlorococcum sp* (0.2 L 8.5 kg/m^3, 2.5 kW, 6 min)	4.50	529	Laboratory, industrial
High Speed Homogenizer	*Saccharomyces cerevisiae* (0.8 L, 10 kg/m^3, 600 W, 15 min)	0.67	67	Laboratory, industrial
Bead mills	*Botryococcus, Chlorella, Scenedesmus* (0.1 L, 5 kg/m^3, 840 W, 5 min)	2.52	504	Laboratory, industrial
Microwave	*Botryococcus, Chlorella, Scenedesmus* (0.1 mL, 5 kg/m^3, 700 W, 5 min)	2.10	420	Laboratory, industrial
Freeze drying	Mathematical modeling on an industrial scale	1.40	140	Laboratory, industrial
Hydrodynamic cavitation	*Saccharomyces cerevisiae* (50 L, 10 kg/m^3, 5.5 kW, 50 min)	0.33	33	Laboratory, pilot scale
Pulsed Electric Field	*Synechocystis* PCC 6803 (5 mL, 0.3 g/L)	0.26	860	Laboratory, pilot scale

Source: adapted from Lee et al. 2012 [16].

On the other hand, the energy required for the indentation and disruption of a single algae cell was estimated as 17 petajoules (pJ) with an atomic force microscope [19], which was estimated to be equivalent to 670 joules (J) per kg of dry algae cell. This clearly shows that the existing cell disruption methods are highly inefficient in transferring energy to the algae cells. In hydrodynamic cavitation, the most "efficient" of the existing methods, only about 0.002% of the energy input was used for cell disruption. This clearly shows that any incremental improvement in the efficiencies of the existing cell disruption methods will not bring about a significant change in the algal biofuel arena. Therefore, an outside the box and transformative solution is necessary for the development of a sustainable algal biofuel industry.

2.3. Project Objectives

The purpose of this project was to develop an energy-efficient, cost-effective, and sustainable microalgae cell disruption method, enhancing lipid extraction. To accomplish this, the following objectives were identified and completed:

- Investigation of the disruption of algae cell with Cu,
- Investigation of the disruption of algae cell with copper sulfate,
- Determination of the increases in lipid extraction yields, and
- Evaluation of the benefits of the project.

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3. CHAPTER 2: Materials and Methods

3.1. Technical Approach

The disruption experiments with Cu and copper sulfate were investigated using: (1) dilute *C. vulgaris* suspension at approximately 0.02% solids, and (2) *C. vulgaris* paste at 10% solids.

3.2. Experimental Set-up for Disruption of *C. vulgaris* Cells in Suspension

The set-up for disruption experiment involving *C. vulgaris* in suspension consisted of 500 mL Erlenmeyer flasks (Kimble Chase) with a taper Polytetrafluoroethylene (PTFE) stopper (Figure 3). The working volume of the reactors was set to 400 ml to maintain semi-batch reactors after periodic withdrawal of samples for various analyses conducted. Amber flasks were used to prevent transmission of light to the algae cells.

Figure 3: Experimental set-up for disruption of algae cells in dilute suspensions

Photo Credit: Biomass Engineering Laboratory, San Diego State University.

In a typical disruption experiment involving *C. vulgaris* in suspension, first the algae culture was centrifuged at 10,000 standard gravity (G) for 10 minutes and the supernatant was discarded. The remaining paste was re-suspended in a phosphate buffered deionized (DI) water at pH of 7.0. This was done to reduce the influence of the residual growth media on Cu or copper sulfate. Then the re-suspended algal biomass was transferred to clean and autoclaved glass bottles. Next, a solution of Cu or copper sulfate was added to the bottles to result in desired doses. The pH of the reactors was adjusted to 7.0 using dilute solutions of 0.1 N of sodium hydroxide (NaOH) or hydrochloric acid (HCl). Then, the reactors were capped, and the contents were mixed continuously using stirrer plates. Samples were periodically collected from the reactors during the course of treatment process. Finally, the cell samples were characterized using cellometer and SEM analyses.

3.3. Experimental Set-up for Disruption of *C. vulgaris* Cells in Paste

The disruption experiment involving *C. vulgaris* paste at 10% was performed in a clean Van Waters & Rogers (VWR) 50-ml centrifuge tubes. To the clean VWR tubes, known mass of the algae paste and a solution of Cu or copper sulfate were added to achieve desired doses of copper and 10% algae cell concentration. The contents of the tubes were mixed using Heidolph Unimax 1010 shaker. Samples were withdrawn periodically and processed for lipid extraction, following the procedure described in Section 3.4.

3.4. Procedure for Lipid Extraction

Multi-phase solvent extraction is the most commonly researched method for extracting lipids from algal biomass. The process involves the use of a solvent that matches the polarity of the target compound, non-polar lipids [20]. The solvent must also make contact with the lipids inside of the cell [21], which generally requires a second polar solvent to break the cell wall and membrane. Several studies [22-27] employed the Bligh and Dyer method [28], which uses chloroform, methanol, and water as co-solvents for extracting and purifying lipids. Additionally, other solvent systems have been investigated as possible extracting solvents, including dichloromethane, methanol, and water [29], dichloromethane and water [30], n-hexane and water [27], ethanol and water [31], and hexane and water [31]. Of the various multi-solvent methods available, the Bligh and Dryer method [28] was the most efficient, resulting in higher lipid yield, and it was used for the extraction of lipid in this project.

During a typical lipid extraction process, 5 g of disrupted algae paste was transferred to 50-ml centrifuge tubes. Next, 10 ml of methanol and 5 ml of chloroform were added to the sample in the centrifuge tubes. Then the content of the tube was mixed for 2 minutes using a Thermolyne Maxi Mix Plus™ vortex (Dubuque, IA). Additional 5 ml of chloroform was added to the sample and the tube was mixed for 30 seconds using the vortex. Finally, 4.5 ml of distilled water was added to the sample and then mixed for 30 seconds using the vortex. The lipid extraction process was performed at room temperature, in the range of 22 to 26˚C.

The mixture was centrifuged using Thermo Scientific Sorvall RC6+ centrifuge (Waltham, MA) at 10,000 G for 15 minutes. This provided complete separation with the mixture of chloroform and lipid layer at the bottom and the methanol and water layer on the top, while the residual algal biomass at the middle (Figure 4). The bottom mixture of chloroform and lipid layer was removed using a glass Pasteur pipette and placed into pre-weighed 125-ml Erlenmeyer flasks. The chloroform was evaporated from the flask using a Heidolph Hei-VAP Precision with glassware set to G5 rotary evaporator, with a bath temperature of 60˚C, pressure of 375 mbar, and rotation speed of 150 revolutions per minute (rpm).

It is anticipated that the residual copper sulfate and Cu would be in the methanol and water mixture layer and/or the residual algal biomass layer. The residual copper sulfate

and Cu, which are inorganic, are very unlikely to partition to the chloroform and lipid layer, which are organic.

Figure 4: Algal lipid extraction at different stages in the extraction process from left to right shows Step 1: after addition of 10 ml of methanol and 5 ml of chloroform; Step 2: after addition of 5 ml of chloroform; Step 3: after addition of 4.5 ml of DI water; and Step 4: after centrifuge and formation of different layers.

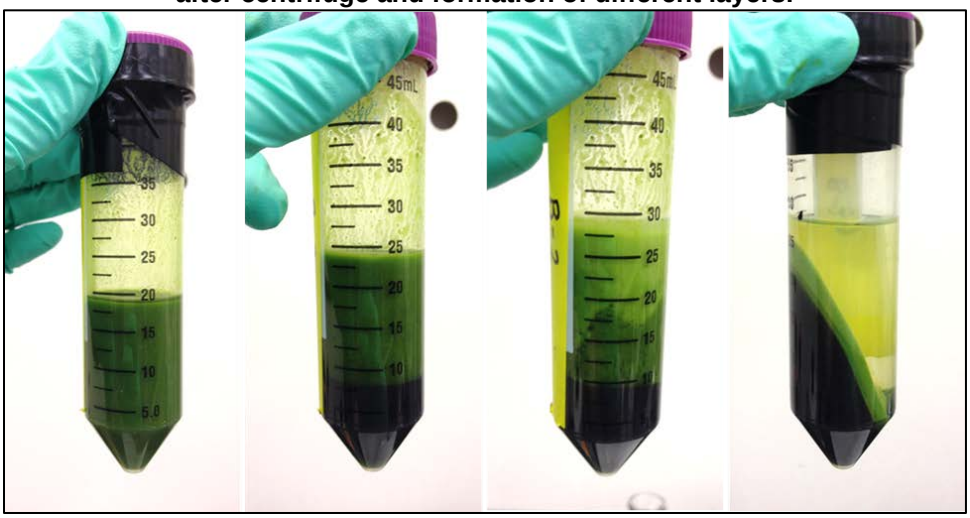


Photo Credit: Biomass Engineering Laboratory, San Diego State University.

3.5. Materials

Chemicals and reagents used in the study were obtained from Fischer Scientific (Pittsburgh, PA) and Sigma-Aldrich Co. (St. Louis, MO). *C. vulgaris*, one of the most widely researched algal species for biofuel feedstock, was used as representative microalgae. *C. vulgaris* culture was purchased from Carolina Biological Supply Company (Burlington, NC).

Cu is available in two forms, granular and dissolved. In preliminary tests, it was observed that granular Cu was slightly miscible with water, while aqueous Cu was completely miscible with water. Algal cells are suspended in growth media where water is the major constituent, and therefore, aqueous Cu was used in this project.

3.5.1 *C. vulgaris* Culture Maintenance, Cultivation and Harvesting

C. vulgaris culture was grown in a medium prepared from MiracleGro All Purpose Water Soluble Plant Food. The media has been used in the past as a simple isolation media for the growth of microalgae in the laboratory [32]. The PI's team also tested mixing rich media with MiracleGro at varying ratios to encourage growth rate and microalgae dominance in culture and determined that the most consistently aseptic and high rate growth came from a minimal media consisting of only MiracleGro solution (2.337 g/L - Hydrated).

During a typical growth cycle (Figure 5), few colonies of *C. vulgaris* from agar-plate cultures were aseptically transferred into 25 ml medium contained in 50 ml VWR tests

tubes, and then capped with sponge plug. The test-tube cultures were placed under fluorescent lighting system, 14 hours' light and 10 hours' dark, and were aerated daily using a vortex. After the culture growth reached to approximately 0.3 to 0.4 abs at 600 nanometer (nm), it was transferred to 500 ml VWR Erlenmeyer flasks containing 350 ml medium. These cultures were aerated using aquarium air pump and were placed on stirrer plates for mixing and under the fluorescent light for 14 days.

Figure 5: A typical *C. vulgaris* biomass production cycle: (a) *C. vulgaris* colony in agar plates, (b) maintenance cultures, (c) starter cultures, (d) growth cultures, and (e) concentrated algal paste

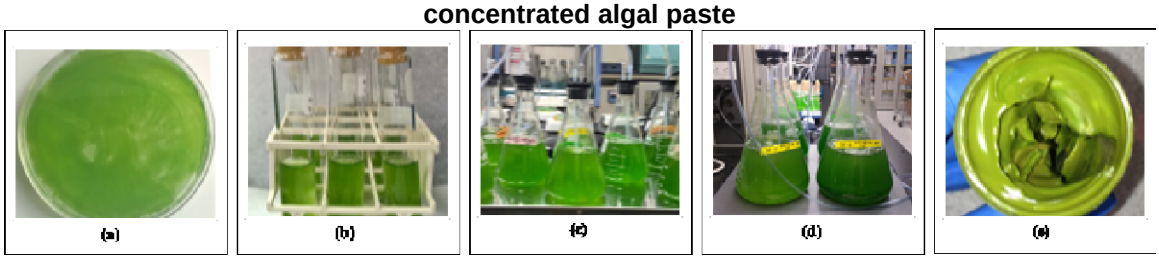
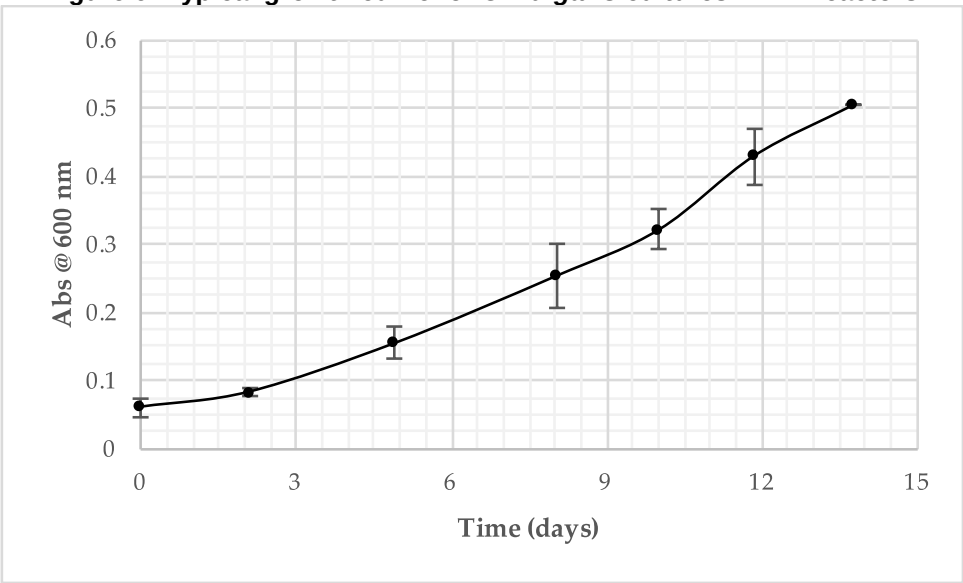


Photo Credit: Biomass Engineering Laboratory, San Diego State University.

Finally, after approximately 0.3 to 0.4 abs at 600 nm was achieved, the cultures were used for the inoculation of 3500 ml medium in 4 L VWR Erlenmeyer flasks. The cultures were aerated with an air stream containing 4.0% CO_2 at a total flow rate of 200 ml/min or 25 ml/min per reactor. Reactors were placed on stirrer plates for mixing and under fluorescent light for 14 days. The cultures were harvested at an absorbance of approximately 0.5 at 600 nm. The cultures were concentrated with centrifugation at 10,000 G for 10 min. A prior study by the project investigator's team has shown that this centrifugation force did not have impact on cell viability [10].

Figure 6 shows a typical growth curve for *C. vulgaris* in for 4 L reactors. The error bars represent one standard error above and below the mean. The figure reveals that the culture passing through lag, accelerated, exponential and stationary growth phases. The cultures are harvested at the onset of the stationary phase, which is around 14 days from the date of inoculation.

Figure 6: Typical growth curve for *C. vulgaris* cultures in 4 L reactors



3.6. Analytical Methods

Algal cell concentration (#cell/ml) and viabilities were determined optically via automated cell counts (Nexcellom Cellometer AutoX4). 20 µl of culture sample was combined with 20 µl propidium iodide (PI) stain (Cellometer ViaStain™ PI Staining Solution) in a 1.5 ml microcentrifuge tube and vortexed for 10 sec. A 20 µl sample was then pipetted to a Cellometer counting chamber and allowed to stabilize for 2 min. A bright field cell was performed, followed by stimulation of the sample at 501 nm and emission measurement at 595 nm for 10 seconds of exposure. Dead cells were identified via fluorescence of PI, and an automated count of fluorescing cells were executed. Percent viability was then determined as the difference between the bright field and fluorescence cell counts divided by the bright field cell count. While cell diameter is directly measured by the Cellometer.

Absorbance at 600 nm was measured with Thermo Scientific BioMate™ 3S Spectrophotometer (Waltham, MA). The pH of the samples was measured using HACH-HQ440d Multi-Parameter Meter with HACH-IntelliCAL-pHC101 probe.

SEM images of the algae cells were obtained using Quanta 450 FEG Scanning Electron Microscope (FEI, OR), housed in San Diego State University's (SDSU) Electron Microscope Facility. For SEM analysis, algae samples treated with copper were fixed with a solution containing 4% (v/v) glutaraldehyde and 0.2 M Sodium Cacadolyic at pH 7.3. The cells were infiltrated by slowly washing with 0.1 M Sodium Cacadolyic at pH 7.3 and dehydrated using a graded ethanol concentration series of 30%, 50%, and 95% ethanol for 10 minutes each. Finally, the cells were soaked in 100% ethanol. A Critical Point Dryer was used to dry the samples further. The samples were then mounted on stubs and coated with platinum using a sputter coater. The morphologies of the surfaces of the ruptured cells were observed by SEM with an accelerating voltage of 5.0 kilovolt (kV).

Lipid separation and analysis was performed using a Hewlett Packard HP 6890 Gas Chromatograph (GC) System and 5973 Mass Selective Detector (MSD). The lipid extracted by the Bligh and Dyer method was transesterified using 2.0% w/w sodium methoxide in methanol, and the fatty acid methyl ester (FAME) was re-extracted using hexane. Then, the sample were manually injected to the GC, and separation was performed on a Thermo Scientific TR-FAME 260M238P column (100 m, 0.25 mm ID, 0.20 µm) with hydrogen at 1.0 mL/minute as the carrier gas. The inlet was set to split less and at 240˚C. The oven was set to 100˚C, hold for 0.2 min, and then increased to 240˚C at 2˚C/min, hold for 15 min. MSD was set with solvent delay of 3.00 minutes and scan and sim mode. The data was further analyzed using automated mass spectral deconvolution and identification system (AMDIS), developed by National Institute of Standard and Technology (NIST). Moreover, a library for FAME identification was created using the Sigma Aldrich FAME Mix 18919-1AMP standard. The list of compounds for which the library was created are presented in Table 2.

Table 2: FAME library based on Sigma Aldrich FAME Mix 18919-1AMP standard

Compound	Formula
11,14,17-Eicosatrienoic acid, methyl	$C_{21}H_{36}O_2$
11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
13-Methyltetra-9-enoic acid, methyl	$C_{15}H_{28}O_2$
15-Tetracosenoic acid, methyl ester	$C_{25}H_{48}O_2$
5,8,11,14,17-Eicosapentaenoic acid,	$C_{21}H_{32}O_2$
6,9,12-Octadecatrienoic acid, methyl	$C_{19}H_{32}O_2$
8,11,14-Eicosatrienoic acid, methyl	$C_{21}H_{36}O_2$
Arachidic acid, methyl ester	$C_{21}H_{42}O_2$
Arachidonic acid, methyl ester	$C_{21}H_{34}O_2$
Capric acid, methyl ester	$C_{11}H_{22}O_2$
Cis-10-Heptadecenoic acid, methyl	$C_{18}H_{34}O_2$
Cis-11,14-Eicosadienoic acid, methyl	$C_{21}H_{38}O_2$
Cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$
Cis-13,16-Docasadienoic acid, methyl	$C_{23}H_{42}O_2$
Cis-4,7,10,13,16,19-Docohexaenoic	$C_{23}H_{34}O_2$
Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$
Elaidic acid, methyl ester	$C_{19}H_{36}O_2$
Erucic acid, methyl ester	$C_{23}H_{44}O_2$
Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$
Lauric acid, methyl ester	$C_{13}H_{26}O_2$
Lignoceric acid, methyl ester	$C_{25}H_{50}O_2$
Linoleic acid, methyl ester	$C_{19}H_{34}O_2$
Linolenic acid, methyl ester	$C_{19}H_{34}O_2$
Margaric acid, methyl ester	$C_{18}H_{36}O_2$
Myristic acid, methyl ester	$C_{15}H_{30}O_2$
Myristoelic acid, methyl ester	$C_{15}H_{28}O_2$
Palmitic acid, methyl ester	$C_{17}H_{34}O_2$
Palmitoleic acid, methyl ester	$C_{17}H_{32}O_2$
Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$
Stearic acid, methyl ester	$C_{19}H_{38}O_2$
Tricosanoic acid, methyl ester	$C_{24}H_{48}O_2$
Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$
Undecanoic acid, methyl ester	$C_{12}H_{24}O_2$

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4. CHAPTER 3: Disruption of *C. vulgaris* Cells with Cu

4.1. Introduction

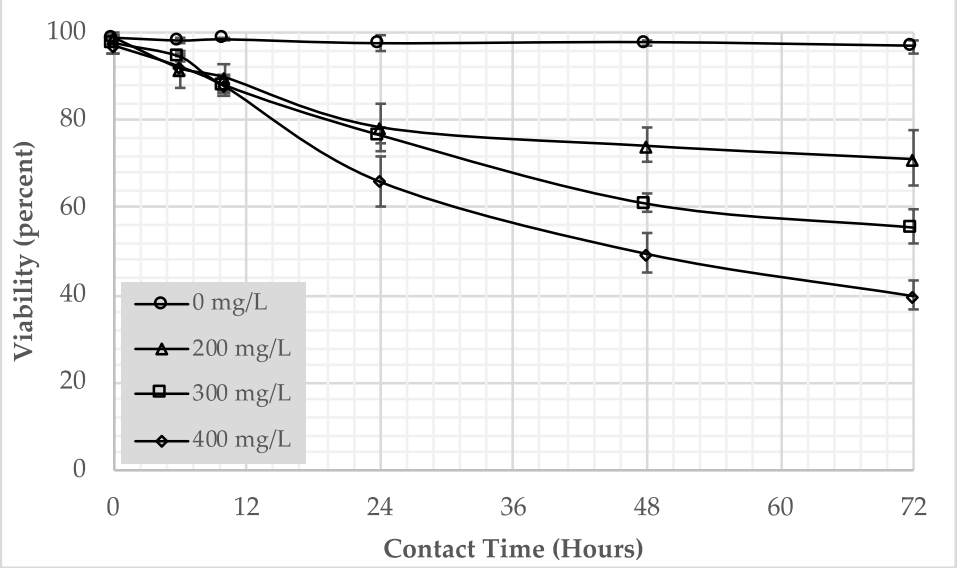
In this chapter, the results obtained from the disruption of *C. vulgaris* cells in suspension with Cu are presented and discussed.

4.2. Disruption of *C. vulgaris* cells in Suspension

Several experiments were conducted to investigate the disruption of *C. vulgaris* cells in suspension. The experimental procedure described in section 3.2 was used. On average, the concentration of algae in the feedstock was approximately 0.02% (approximately 0.2 g/L) on dry mass basis. The concentration on the basis of number of cells was determined as 8.50 ± 1.11 x 10^6 cells/mL. The samples were dosed at 0 (control), 200, 300, and 400 mg/L of Cu as Cu. Samples were collected from the reactors during the course of treatment process at 0, 6, 10, 24, 48, and 72 hours and were analyzed for cell variability using Nexcellom Cellometer AutoX4. Refer to Section 3.6 for details on the analytical procedures.

The results from the cellometer analysis are presented in Figure 7. The data represents mean values from sextuplicate analyses, from triplicate reactors, with one standard deviation above and below the mean.

Figure 7: Algae cell viability as a function of contact time and varying Cu dose

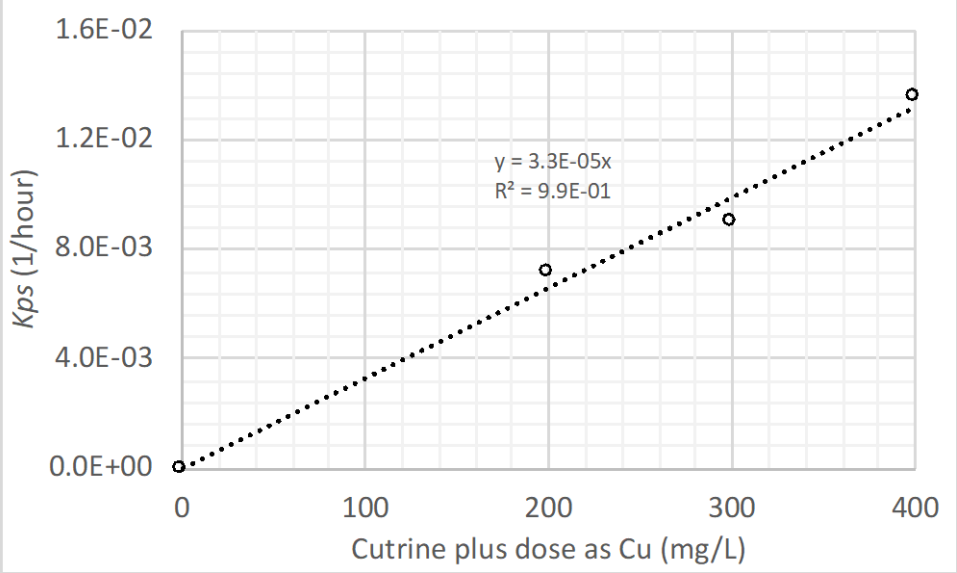


The results revealed that the cell viability for the reactors dosed with 0 mg/L of Cu (controls) remained constant at approximately 100% over the course of the treatment process, as expected, while the cell viabilities decreased for samples dosed with 200, 300, and 400 mg/L Cu as Cu. At the end of the 72-hour contact time, the cell

variabilities decreased to 71%, 56%, and 40% for samples dosed with 200, 300, and 400 mg/L Cu as Cu, respectively; indicating that cell disruption increases with Cu dose.

The pseudo first-order rate constants (k_{ps}) for cell disruption were estimated as 7.2 x10^{-3}, 9.0 x 10^{-3}, and 1.4 x 10^{-2} $hour^{-1}$ for samples dosed with 200, 300, and 400 mg/L Cu as Cu, respectively. The intrinsic rate constant (k) for the disruption of *C. vulgaris* cells in suspension with Cu followed first-order with respect copper dose (Figure 8), and it was estimated as 3.3 x 10^{-5} L/mg $hour^{-1}$.

Figure 8: Estimation of the intrinsic rate constant of the disruption of *C. vulgaris* with Cu



After treatment with Cu, the surfaces of the ruptured cells were analyzed with SEM. The morphologies of the surfaces are shown in Figure 9. Figure 9a shows the morphology of the *C. vulgaris* cell surface without treatment, from control reactors. Figure 9b shows the morphologies of C. *vulgaris* cells treated with Cu, illustrating the broken appearance of the cells into irregular shapes, compared to the spherical for non-treated (control) cells in Figure 9a. In addition, cells treated with Cu exhibited uneven shrinkages and creases. The SEM morphologies of the cell surfaces confirm that the walls of the C. *vulgaris* cells were ruptured after treatment with Cu. Moreover, the SEM morphologies also revealed that some complete cells remained after treatment (images not shown), confirming the results of cell variability analyses presented earlier.

Figure 9: Scanning electron micrographs of the *C. vulgaris* cell surfaces: (a) cell without treatment (control) and (b) cells treated with Cu; scale bars denote 1 μm

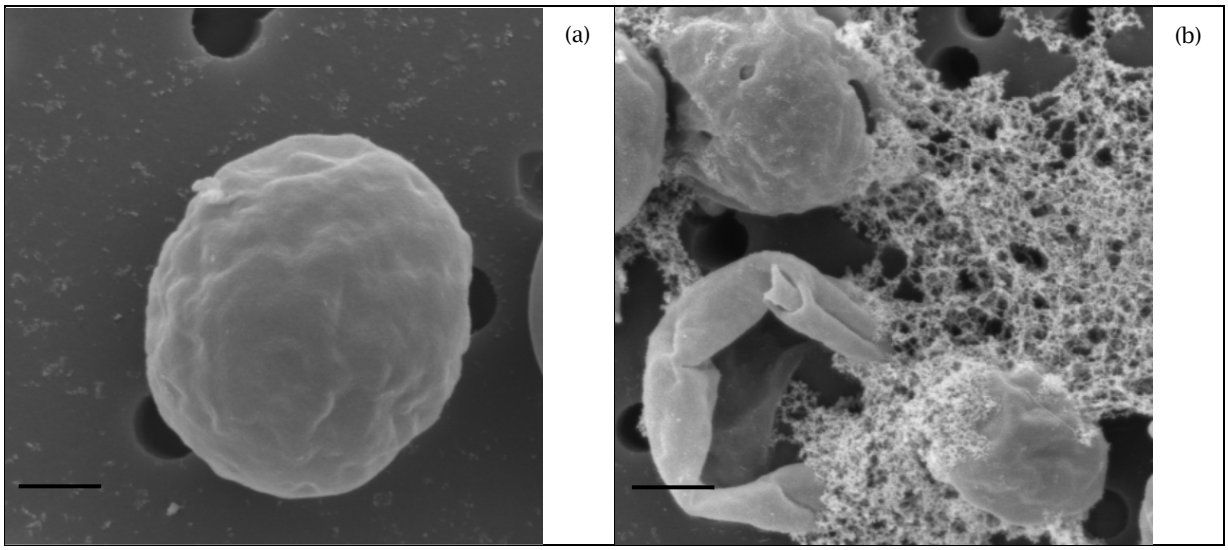


Photo Credit: Biomass Engineering Laboratory, San Diego State University.

4.3. Summary

The results presented in this chapter showed that Cu was effective in disrupting algae cells. Cellometer and SEM analyses of the samples confirmed that the algae cells were ruptured after treatment with Cu. Cell viabilities decreased with increase in copper dose. At the end of 72-hour contact time, the cell variabilities were measured as 71%, 56%, and 40% for samples dosed with 200, 300, and 400 mg/L Cu as Cu, respectively. The pseudo first-order rate constants for cell disruption were estimated as 7.2×10^{-3}, 9.0×10^{-3}, and 1.4×10^{-2} $hour^{-1}$ for samples dosed with 200, 300, and 400 mg/L Cu as Cu, respectively. The intrinsic rate constant for the disruption of *C. vulgaris* cells in suspension with Cu followed first-order with respect copper dose, and it was estimated as 3.3×10^{-5} L/mg $hour^{-1}$.

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5. CHAPTER 4: Disruption of *C. vulgaris* Cells with Copper Sulfate

5.1. Introduction

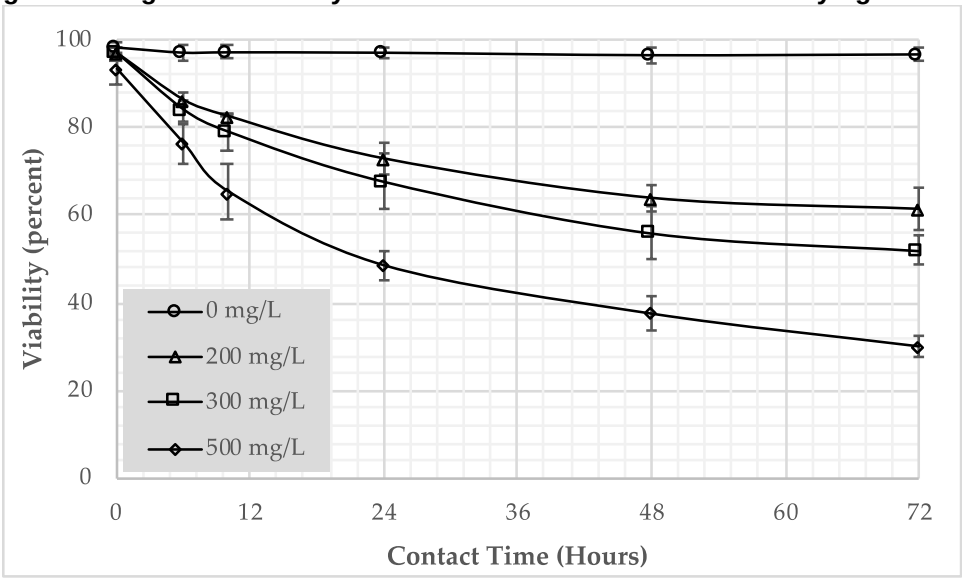
In this chapter, the results obtained from the disruption of *C. vulgaris* cells in suspension with copper sulfate are presented and discussed.

5.2. Disruption of *C. vulgaris* cells in Suspension

Several experiments were conducted to investigate the disruption of *C. vulgaris* cells in suspension. The experimental procedure described in section 3.2 was used. On average, the concentration of algae in the feedstock was approximately 0.02% (approximately 0.2 g/L) on dry mass basis. The concentration on the basis of number of cells was determined as 5.91 ± 0.97 x 10^6 cells/ml. The samples were dosed at 0 (control), 200, 300, and 500 mg/L of copper sulfate as Cu. Samples were collected from the reactors during the course of treatment process at 0, 6, 10, 24, 48, and 72 hours and were analyzed for cell variability using Nexcellom Cellometer AutoX4. Refer to section 3.6 for details on the analytical procedures.

The results from the cellometer analysis are presented in Figure 10. The data represents mean values from sextuplicate analyses, from triplicate reactors, with one standard deviation above and below the mean.

Figure 10: Algae cell viability as a function of contact time and varying Cu dose

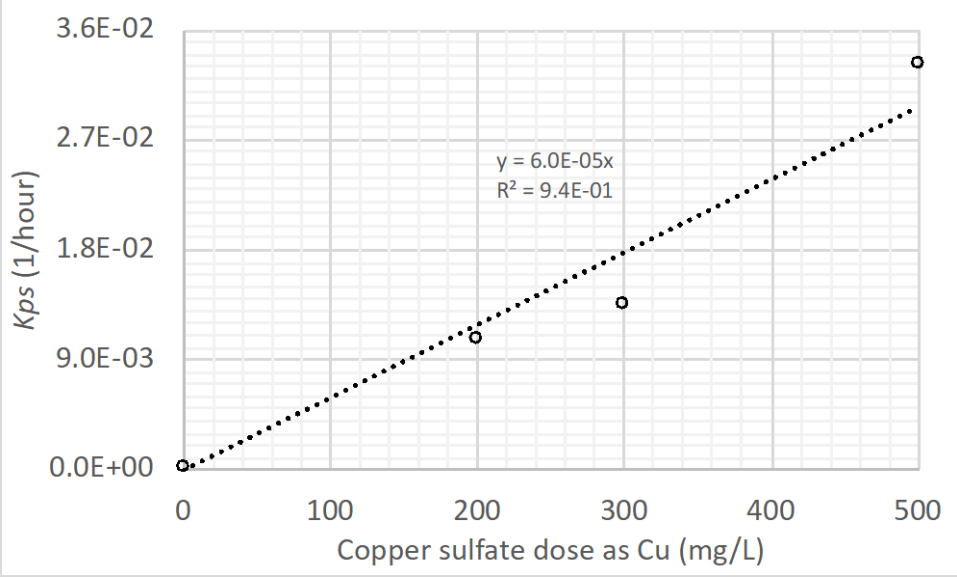


The results revealed that the cell viability for the reactors dosed with 0 mg/L of Cu (controls) remained constant at approximately 100% over the course of the treatment process, as expected, while the cell viabilities decreased for samples dosed with 200,

300, and 500 mg/L copper sulfate as Cu. At the end of the 72-hour contact time, the cell variabilities decreased to 62%, 52%, and 30% for samples dosed with 200, 300, and 500 mg/L copper sulfate as Cu, respectively; indicating that cell disruption increases with copper sulfate dose.

The pseudo first-order rate constants for cell disruption were estimated as 1.1 x10^{-2}, 1.3 x 10^{-2}, and 3.3 x 10^{-2} $hour^{-1}$ for samples dosed with 200, 300, and 500 mg/L copper sulfate as Cu, respectively. The intrinsic *k* for the disruption of *C. vulgaris* cells in suspension with copper sulfate followed first-order with respect copper dose (Figure 11), and it was estimated as 6.0 x 10^{-5} L/mg $hour^{-1}$.

Figure 11: Estimation of the intrinsic rate constant of the disruption of *C. vulgaris* with copper sulfate



After treatment with copper sulfate, the surfaces of the ruptured cells were analyzed with SEM. The morphologies of the surfaces are shown in Figure 12. Figure 12a shows the morphology of the *C. vulgaris* cell surface without treatment, from control reactors. Figure 12b shows the morphologies of C. *vulgaris* cells treated with copper sulfate, illustrating the broken appearance of the cells into irregular shapes, compared to the spherical for non-treated (control) cells in Figure 12a. In addition, cells treated with copper sulfate exhibited uneven shrinkages and creases. The SEM morphologies of the cell surfaces confirm that the walls of the C. *vulgaris* cells were ruptured after treatment with copper sulfate. Moreover, the SEM morphologies also revealed that some complete cells remained after treatment (images not shown), confirming the results of cell variability analyses presented earlier.

Figure 12: Scanning electron micrographs of the *C. vulgaris* cell surfaces: (a) cell without treatment (control) and (b) cells treated with copper sulfate; scale bars denote 1 μm

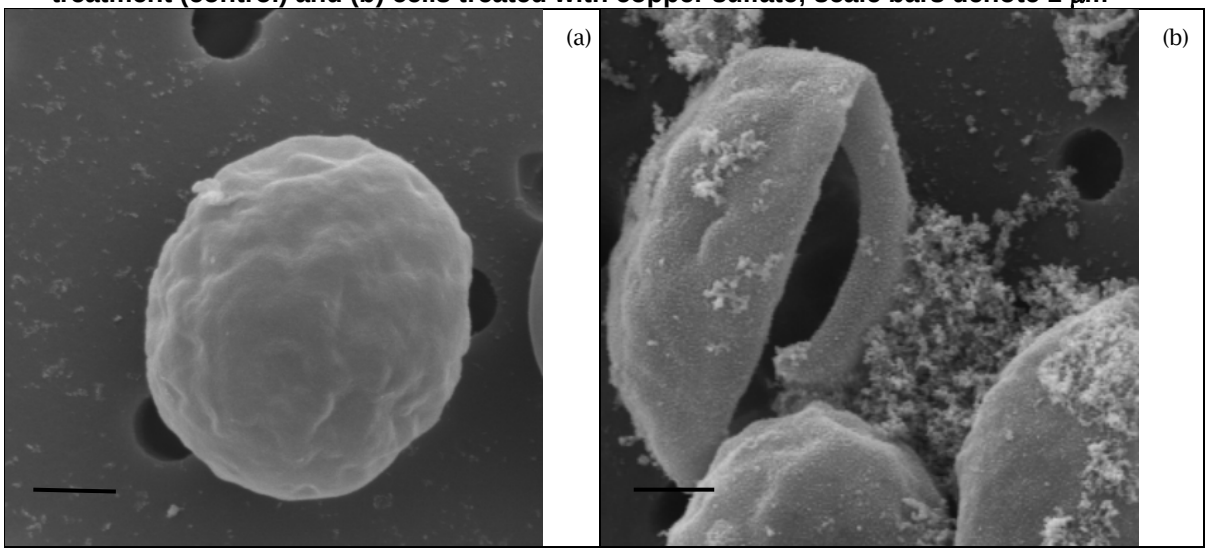


Photo Credit: Biomass Engineering Laboratory, San Diego State University.

5.3. Summary

The results presented in this chapter showed that copper sulfate was effective in disrupting algae cells. Cellometer and SEM analyses of the samples confirmed that the algae cells were ruptured after treatment with copper sulfate. Cell viabilities decreased with increase in copper dose. At the end of 72-hour contact time, the cell variabilities were measured as 62%, 52%, and 30% for samples dosed with 200, 300, and 500 mg/L copper sulfate as Cu, respectively. The pseudo first-order rate constants for cell disruption were estimated as 1.1 $x10^{-2}$, 1.3 x 10^{-2}, and 3.3 x 10^{-2} $hour^{-1}$ for samples dosed with 200, 300, and 500 mg/L copper sulfate as Cu, respectively. The intrinsic rate constant for the disruption of *C. vulgaris* cells in suspension with copper sulfate followed first-order with respect copper dose, and it was estimated as 6.0 x 10^{-5} L/mg $hour^{-1}$.

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6. CHAPTER 5: Lipid Extraction Yields

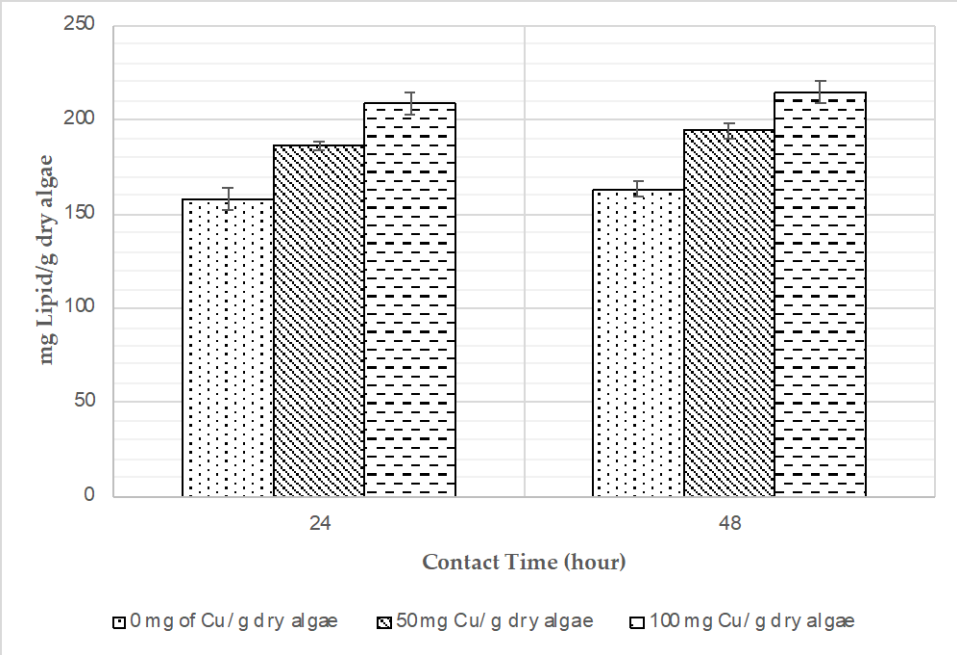
6.1. Introduction

In this chapter, the results obtained from the extraction of lipid from algal biomass disrupted with Cu and copper sulfate are presented and discussed.

6.2. Lipid Extraction from Algal Biomass Disrupted with Cu

Disruption and lipid extraction experiments were conducted using C. *vulgaris* paste at 10% solids dosed with 0 (controls), 50, and 100 mg of Cu as Cu per g of dry algae. The experimental procedure described in Section 3.4 was used. Samples were collected at 24 and 48 hours, and then lipid extraction was performed on them. The results as mg of lipid extracted per g of dry algae are presented in Figure 13. The data represents mean values from triplicates with one standard deviation above and below the mean.

Figure 13: Lipid extraction as a function of contact time and Cu dose



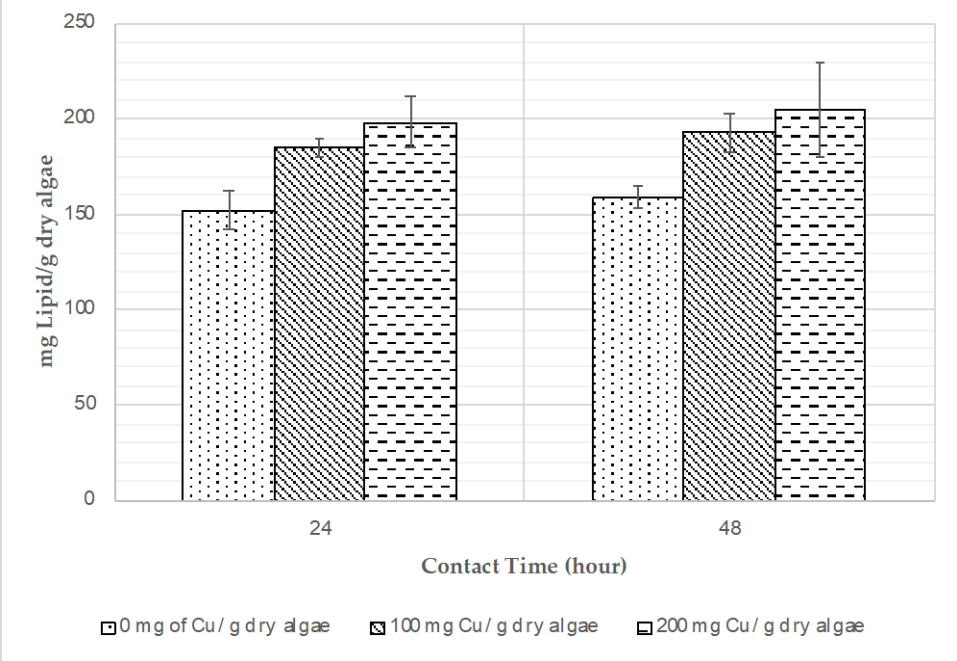
The data revealed that lipid extraction increased with copper dose. For control samples, as expected, the lipid extraction yield remained constant around 160 mg of lipid per g of *C. vulgaris* after 24- and 48-hour contact times. For samples dosed with 50 mg of Cu as Cu per g of dry *C. vulgaris*, lipid extraction yields of 186 and 194 mg per g of *C. vulgaris* were achieved at 24- and 48-hour contact times, respectively. Lipid extraction yields of 208 and 215 mg per g of *C. vulgaris* were achieved at 24- and 48-hour contact times, respectively, for samples dosed with 100 mg of Cu as Cu per g of dry *C. vulgaris*.

The *p*-values, at an α of 0.05, for various paired *t*-tests for lipid extraction yield achieved at different copper doses (0 vs. 50 mg/g, 0 vs. 100 mg/g, and 50 vs. 100 mg/g) were < 0.01, indicating that there were significant differences for the yields. On the other hand, there was no significant difference in lipid yields for samples dosed with 0, 50, and 100 mg of Cu as Cu per g of dry algae for paired *t*-tests at contact times of 24 and 48 hours; the *p*-values for the paired *t*-test were > 0.05.

6.3. Lipid Extraction from Algal Biomass Disrupted with Copper Sulfate

Similarly, disruption experiments were conducted using C. *vulgaris* paste at 10% solids dosed with 0 (controls), 100, and 200 mg of copper sulfate as Cu per g of dry algae. The experimental procedure described in section 3.4 was used. Samples were collected at 24 and 48 hours, and then lipid extraction was performed on them. The results as mg of lipid extracted per g of dry algae are presented in Figure 14. The data represents mean values from triplicates with one standard deviation above and below the mean.

Figure 14: Lipid extraction as a function of contact time and copper sulfate dose



The data revealed that lipid extraction increased with copper dose. For control samples, as expected, the lipid extraction yield remained constant around 155 mg of lipid per g of *C. vulgaris* after 24- and 48-hour contact times. For samples dosed with 100 mg of copper sulfate as Cu per g of dry *C. vulgaris*, lipid extraction yields of 185 and 193 mg per g of *C. vulgaris* were achieved at 24- and 48-hour contact times, respectively. Lipid extraction yields of 200 and 205 mg per g of *C. vulgaris* were achieved at 24- and 48-hour contact times, respectively, for samples dosed with 200 mg of copper sulfate as Cu per g of dry *C. vulgaris*.

The *p*-values, at an α of 0.05, for paired *t*-tests for lipid extraction yield achieved at 0 and 100 and 0 and 200 mg of Cu per g of dry algae were < 0.01, indicating that there were significant differences for the yields. On the other hand, the *p*-value for paired *t*-test for lipid extraction yield achieved at 100 and 200 mg of Cu per g of dry algae was > 0.3, indicating that there was no significant difference. Moreover, there was no significant difference in lipid yields for samples dosed with 0, 100, and 200 mg of copper sulfate as Cu per g of dry algae for paired *t*-tests at contact times of 24 and 48 hours; the *p*-values for the paired *t*-test were > 0.4.

6.4. Comparison of Lipid Extraction Yields

In this section, the increase in lipid extraction yield achieved in this project was compared with yields reported in the literature for some of the current algal cell disruption methods (refer to section 2.2 for the summary of current disruption technologies). The percent increase in the lipid extraction yield was estimated using the following equation:

$$PIY = \frac{(LEY_{pret} - LEY_{ctrl})}{LEY_{ctrl}} * 100$$

Where *PIY* is the percent (%) increase in lipid extraction yield, LEY_{pret} is the lipid extraction yield (in mass of lipid/mass of dry algae) for algal biomass pretreated with cell disruption methods, and LEY_{ctrl} is the lipid extraction yield (in mass of lipid/mass of dry algae) for control (untreated) samples.

In this study, increases in lipid extraction yields of 18% to 32% were achieved for samples pretreated with Cu or copper sulfate compared to controls. The percent increases were in the lower end of those reported for other cell disruption methods. The pretreatment of algal biomass with pulse electric field has been reported to increase lipid extraction by as low as 5.3% [10] and as high as 150% [33] compared to control samples. The values reported for percent increases in lipid extraction compared to untreated samples ranged from 30% [25] to 120% [23] for ultra-sonication and from 40% [29] to 600% [30] for microwaves.

A majority of these studies used different solvent ratios that may not have been as ideal as those prescribed by Bligh and Dyer [28], and the benefits of pretreatment may have been greater for less efficient solvents. Additionally, most of the studies included additional sample processing before pretreatment and extraction, including freeze drying and pretreating the sample with saline solution, which can disrupt the cells and can enhance the actual cell disruption. Some of the studies used ethyl acetate as an extracting solvent [33], which is less efficient than chloroform, so the benefits of pretreatment may have been greater. Refer to section 3.4 for description of multi-solvent lipid extraction methods.

6.5. Effect of Pretreatment on Lipid Profile

The effect of algal biomass pretreatment with Cu or copper sulfate on lipid profile was investigated using GC-MSD analysis. Lipids obtained from algal biomass treated with Cu or copper sulfate and control samples (untreated) were transesterified, and then analyzed and compared. The same lipid extraction and lipid transesterification processes were used for the control and samples pretreated with Cu and copper sulfate.

The transesterified lipid compounds identified using the NIST and FAME libraries are presented in Table 3. Refer to Section 3.6 for details on the analytical procedures. The result showed that there were no major differences in the lipid profiles between controls and samples pretreated with copper sulfate after 24 or 48 hours of contact time. For Cu, few compounds were not detected at 24 hours contact time, but at 48-hours contact time, the lipid profiles of the control and Cu pretreated samples were similar. Therefore, it can be inferred that, pretreatment of algal biomass with Cu or copper sulfate has no influence on the lipid profiles, hence the biodiesel that would be produced from the resulting lipids.

Table 3: Transesterified lipid compounds identified

Transesterified Lipid Compounds		Time		100 mg Cu as Cu/g dry algae		100 mg copper sulfate as Cu/g dry algae	
		24-hr	48-hr	24-hr	48-hr	24-h4	48-hr
Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	√	√	√	√	√	√
7,10- Hexadecadienoic acid,	$C_{17}H_{30}O_2$	√	√	√	√	√	√
7,10,13- Hexadecatrienoic	$C_{17}H_{28}O_2$	√	√	ND	√	√	√
Stearic acid, methyl ester	$C_{19}H_{38}O_2$	√	√	√	√	√	√
Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	√	√	√	√	√	√
Linolenic acid, methyl ester	$C_{19}H_{34}O_2$	√	√	√	√	√	√
Myristic acid, methyl ester	$C_{15}H_{30}O_2$	√	√	ND	√	√	√
Myristoleic acid, methyl ester	$C_{15}H_{28}O_2$	√	√	ND	ND	ND	√
Pentadecanoic acid, methyl	$C_{16}H_{32}O_2$	√	√	ND	√	√	√
Palmitoleic acid, methyl	$C_{17}H_{32}O_2$	√	√	ND	√	√	√
cis-10-Heptadecenoic acid,	$C_{18}H_{34}O_2$	ND	ND	ND	√	ND	√
11-Octadecenoic acid,	$C_{19}H_{36}O_2$	√	√	√	√	√	√

ND – Not Detected

√ – Detected

6.6. Summary

The results presented in this chapter showed the disruption of *C. vulgaris* cells with Cu and copper sulfate increased lipid extraction yields. For Cu, the lipid extraction yield

was improved by as high as 32% with respect to control samples for 10% algae paste dosed with 100 mg of Cu as Cu per g of dry algae and 24-hours of contact time. About 30% increase in lipid yield was achieved for copper sulfate at 200 mg of Cu per g of dry algae.

The percent increases achieved in this project were in the lower end of those reported for other cell disruption methods. A majority of the studies reported in the literature used different solvent ratios that may not have been as ideal as those prescribed by Bligh and Dyer, and the benefits of pretreatment may have been greater for less efficient solvents. Additionally, most of the studies included additional sample processing before pretreatment and extraction, including freeze drying and pretreating the sample with saline solution, which can disrupt the cells and can enhance the actual cell disruption. Some of the studies used ethyl acetate as an extracting solvent, which is less efficient than chloroform, so the benefits of pretreatment may have been greater.

The results from GC-MSD analysis of the transesterified lipid showed that there were no major differences in lipid profiles between controls and samples pretreated with Cu or copper sulfate. Therefore, it was inferred that pretreatment of algal biomass with Cu or copper sulfate has no influence on the lipid profiles, hence the biodiesel that would be produced from the resulting lipids.

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7. CHAPTER 6: Project Benefits

7.1. Introduction

In the previous chapters, the feasibility of using Cu and copper sulfate as algal cell disruption agents was investigated. The results revealed that Cu and copper sulfate were effective in disrupting *C. vulgaris*, the representative microalgae used in this project. In this chapter, the benefits of using Cu and copper sulfate as algal biomass disruption methods were evaluated. In evaluating the benefits, parameters, such as energy-efficiency, cost-effectiveness, and sustainability of the proposed methods compared with existing methods, were employed. First, the energy input and GHG emissions during the production of Cu and copper sulfate were estimated. Next, the specific energy requirement and GHG emission per mass of algal biomass disrupted by using Cu and copper sulfate were estimated, and the values were compared with values reported in the literature for existing algal biomass disruption methods. The cost-effectiveness of the proposed methods compared with existing algal cell disruption methods was evaluated on the basis of operating cost since the capital cost for the existing methods were not readily available. Finally, the benefits of adapting the technology in California were assessed.

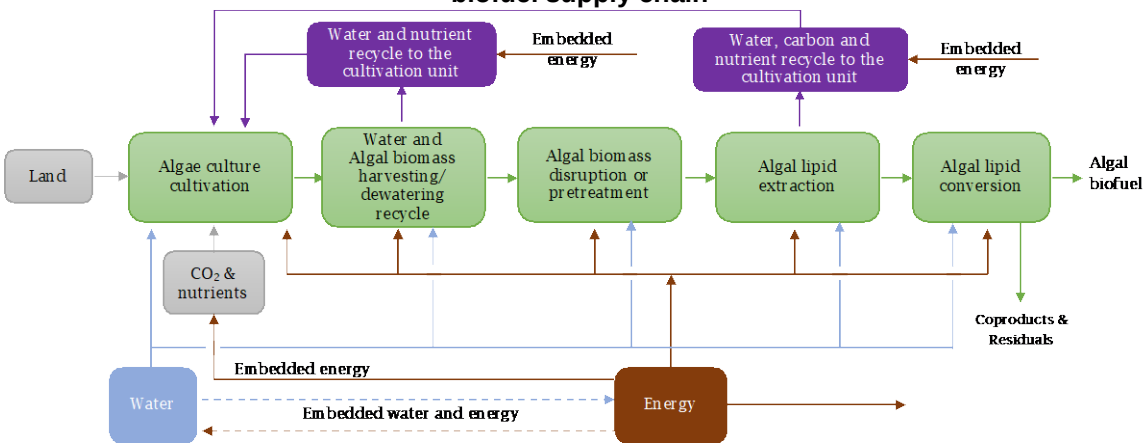
7.2. Algal Biofuel Supply Chain

As stated in Chapter 2, currently two approaches are pursued in the algae-to-biofuel pathway: (1) algal lipid extraction and upgrading, and (2) whole algae hydrothermal liquefaction and upgrading. In the former pathway, microalgae cultivation, harvesting/ dewatering, disruption/ pretreatment, extraction, and conversion are the main steps involved.

In this section, a high-level of algal biofuel production and supply chain for the lipid extraction and upgrading pathway is briefly discussed. Algal biofuel production system requires a number of inputs – land, water, carbon source, and energy, to mention a few of them. Figure 15 provides a simplified high-level overview of the key resources required in the algal biofuel supply chain. The cultivation step requires most of the inputs – land, water, CO_2 as carbon source, nutrients, and energy. On the other hand, energy and water are the major input to the other process units – harvesting/ dewatering, disruption/ pretreatment, extraction, and conversion. Land is required as inputs to these units, but insignificant compared to the requirements for the cultivation steps. Moreover, the water usage rate for the cultivation step depends whether open pond or PBR growth systems are employed, and this will be discussed in Section 7.7.4 in detail.

It should also be noted that the resources presented in the Figure 15 are not exhaustive. Additional resource such as materials, capital, labor, and other inputs associated with the construction, operations, and maintenance of the facilities are needed.

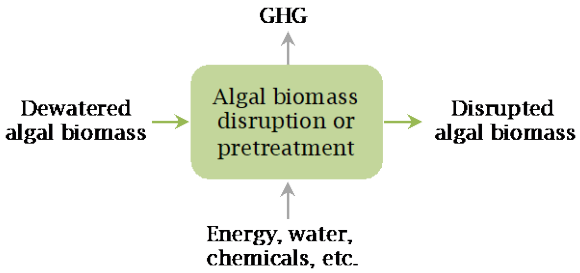
Figure 15: A simplified high-level overview of the key resources required in the algal biofuel supply chain



7.3. Baseline for Algal Biomass Disruption Technologies

The focus of this project was to develop new methods for algal biomass disruption or pretreatment methods. As presented in Section 2.2, a variety of disruption methods are currently available for algal biomass disruption, including bead milling, high-pressure homogenization, high-speed homogenization, hydrodynamic cavitation, microwave, ultra-sonication, pulsed electric field, among others field [14, 16, 34-38]. To compare the methods developed in this project with current methods, resources input into the unit and associated environmental issues are identified in Figure 16. Energy is the key resource required for all the disruption methods. In addition, the methods developed in this project require the addition of chemicals. The major output from all the methods associated with environmental issues is the emission of GHG.

Figure 16: Key resources input into and associated environmental issues resulting from algal biomass disruption methods



Like the algae-to-biofuel pathway supply chain, additional resource such as capital, labor, and other inputs associated with the construction, operations, and maintenance of the disruption technologies are needed. However, such a detailed resource accounting and analysis is not warranted at this stage of the technology. Therefore, energy-efficiency, cost-effectiveness, and sustainability, as measured by GHG emission, of Cu and copper sulfate as potential algal cell disruption agents were estimated and compared with those of the existing methods. A brief description of the cell disruption methods is provided in Table 4.

Table 4: A brief description of the current cell disruption methods

Cell Disruption Methods	Process Description	Mechanism of Cell Disruption
Hydrodynamic cavitation	Works by subjecting cell suspensions to rapid changes of pressure that cause the formation of cavities in the liquid when the pressure is relatively low and then the voids implode at higher pressure, generating intense shock wave which disrupts the cells.	Cavitation and shear stress
High-speed homogenizer	Consists of a stator–rotor assembly when stirred at high speed, creating cavitation due to a local pressure drop nearly down to the vapor pressure of the liquid. Subsequently, as the liquid moves away from the impeller, the liquid pressure restores proportional to the decrease in velocity and the distance from impeller tip and causes the collapse of the cavities. As the cavities collapse, they generate intense shock wave which disrupts the cells.	Cavitation and shear stress
Sonication	The process of applying high frequency acoustic waves that initiate a cavitation process and a propagating shock wave forms jet streams in the surrounding medium causing cell disruption by high shear forces and free radicals.	Cavitation and free radicals
Freeze drying	Freeze drying is the process of removing water from materials through a low temperature dehydration process which involves freezing the product, lowering pressure, then removing the ice by sublimation.	Thermal and osmotic shock
Microwave	A suspension is exposed to microwaves, the microwaves interact selectively with the dielectric or polar molecules (e.g., water) and cause local heating as a result of frictional forces from inter- and intramolecular movements. Water exposed to microwaves reaches the boiling point fast resulting in expansion within the cell and an increase in the internal pressure. The local heat and pressure combined with the microwave induced damage to the cell membrane/wall, facilitates the release of cell contents.	Temperature increase, molecular energy increase

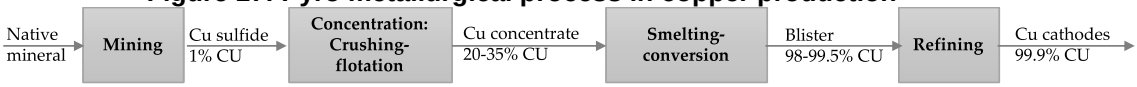
Cell Disruption Methods	Process Description	Mechanism of Cell Disruption
Bead mills	Employ very small glass, ceramic or steel beads placed in a vessel along with the sample media. Disruption of the sample occurs as the beads collide rapidly with the cells, induced by vigorous agitation of the vessel. After the processing cycle is complete, the beads settle by gravity and separated from homogeneous cell materials.	Mechanical compaction and shear stress
High-pressure homogenizer	Work by forcing cell suspensions through a very narrow channel or orifice under pressure. Cell disruption is achieved through high-pressure impact of the accelerated fluid jet on the stationary surface as well as hydrodynamic cavitation from the pressure drop induced shear stress.	Cavitation and shear stress
Pulsed electric field	Uses an external electric field to induce an electrical potential across the cell wall. Cell disruption is caused by electromechanical compression and electric field-induced tension inducing pore formation in the wall.	Proliferation due to electricity
Cu, copper sulfate (this study)	The cell disruption agents are brought in contact with cells in suspension in a reactor or vessel by mixing. The agents disrupt the cells by interaction with the cell membrane components or cell contents.	Chemical substrate interaction

7.4. Energy Input and GHG Emissions during Copper Sulfate Production

Copper sulfate is produced industrially by the reaction between copper metal and hot concentrated sulfuric acid. Thus, the production of copper sulfate can be divided into three parts: 1) the production of copper, 2) the production of sulfuric acid, and 3) the production of copper sulfate.

The basic steps in the manufacturing of copper involve copper ore mining, copper processing, and copper benefaction, i.e., washing, crushing and grinding. Copper can be extracted from three types of ores: sulfide, oxide and native metal ores. Sulfide ores are generally mined with help of pyro metallurgical process as the iron content can be used to obtain the copper concentrate. Here, the sulfide ore is crushed and concentrated, later smelted and then blistered to obtain approximately 98% of copper, Figure 17.

Figure 17: Pyro metallurgical process in copper production



Source: Alvaradoa et al., 1999 [39]

In oxide ores, the copper extraction is performed with the help of hydrometallurgical processes. In this process, oxide ores are leached with acid, concentrated and purified by solvent extraction and then pure copper is recovered by electro winning process.

It was estimated that mining, concentration, smelting, and refining consume about 20%, 50%, 17%, and 13%, respectively, of the overall energy requirement for copper production [39]. From the review of various studies reported in the literature [40-42], the energy required in the copper pyro metallurgical process was approximately 35 MJ/kg of copper (2.24 MJ/mole of copper). The GHG emission was estimated as 3.3 kg CO_2-eq/kg of copper (0.21 kg CO_2-eq/mole of copper) [42].

The production of one mole of copper sulfate requires one mole of copper and one mole of sulfuric acid. Therefore, data on the energy requirement and GHG emission for sulfuric acid production is necessary. According to the European Sulfuric Acid Association [43], the total energy requirement and GHG emission were estimated to be 0.18 MJ/kg (1.76×10^{-2} MJ/mole) and 0.15 kg of CO_2-eq/kg (1.45×10^{-2} kg CO_2-eq /mole of copper), respectively.

Thus, the production of one mole of copper sulfate requires an input of 2.24 MJ/mole (for copper) plus 1.76×10^{-2} MJ/mole (for sulfuric acid) = 2.2576 MJ/mole of copper sulfate. This is equivalent to 14.11 MJ/ kg of copper sulfate. This is value in agreement with the value reported as 15 MJ/kg of copper sulfate by Adom and Dunn, 2015 [44].

Similarly, the GHG emission per mole of copper sulfate was estimated as 0.21 kg CO_2-eq/mole (for copper) plus 1.45×10^{-2} CO_2-eq /mole (for sulfuric acid) = 0.2259 kg CO_2-

eq/mole, which is equivalent to 1.41 kg CO_2-eq/kg. Again, the value is in agreement with the value reported as 1.4 kg CO_2-eq/kg of copper sulfate by Adom and Dunn, 2015 [44].

7.5. Energy Input and GHG Emissions during Cu Production

The production of Cu involves the complexation of copper ion (Cu^{2+}) and ethanolamine (C_2H_7NO). On the other hand, the production of ethanolamine takes place by the reaction between ammonia (NH_3) and ethylene oxide (C_2H_4O). Thus, the production of Cu can be divided into five parts: 1) the production of copper, 2) the production of ammonia, 3) the production of ethylene oxide, 4) the production of ethanolamine, and 5) the production of Cu. In the following subsections, the estimated energy input and CO_2 emission during the production of these chemicals are presented.

7.5.1 The Production of Copper

In the previous subsection, the processes involved in the production of copper were described. Accordingly, the total energy requirement for copper production was estimated as 35 MJ/kg of copper (2.24 MJ/mole of copper) and the GHG emission was equivalent to 3.3 kg CO_2-eq/kg of copper (0.21 kg CO_2-eq/mole of copper).

7.5.2 The Production of Ammonia

Ammonia is synthesized from nitrogen (N_2) and hydrogen (H_2) by the following reaction:

$$N_2 + 3H_2 \rightarrow 2NH_3$$

Nitrogen available in the atmospheric air is the best source of nitrogen while hydrogen can be produced from various feedstocks, although currently it is derived mostly from fossil fuels through either steam reforming or partial oxidation process, depending on the type of fossil fuel. However, about 80% of the ammonia production capacity worldwide is currently provided by the well-developed steam reforming process using natural gas as feedstock [45].

The total energy requirement for ammonia production was estimated as 15.38 MJ/kg of ammonia (0.26 MJ/mole of ammonia) and the GHG emission was equivalent to 2.52 kg CO_2-eq/kg of ammonia (0.043 kg CO_2-eq/mole of ammonia) [46].

7.5.3 The Production of Ethylene Oxide

Ethylene oxide is industrially produced by oxidation of ethylene (C_2H_4) in the presence of silver catalyst. The total energy requirement for ethylene oxide production was estimated as 10 MJ/kg (0.44 MJ/mole) and the GHG emission was equivalent to 1.16 kg CO_2-eq/kg (0.051 kg CO_2-eq/mole) [46].

7.5.4 The Production of Ethanolamine

Ethanolamine is produced by reacting ethylene oxide with aqueous ammonia as follows:

$$C_2H_4O + NH_3 \rightarrow C_2H_7NO$$

This reaction is exothermic, and thus requires no energy input. Therefore, the energy input and CO_2 emission during the production of ethanolamine can be estimated using the energy consumed and CO_2 emitted during the production of ethylene oxide and ammonia. Accordingly, the energy input was estimated as 0.7 MJ/mole of ethanolamine (11.48 MJ/kg of ethanolamine) and the CO_2 emission was estimated as 0.094 kg CO_2-eq/mole of ethanolamine (1.54 kg CO_2-eq/kg of ethanolamine).

7.5.5 The Production of Cu

The energy consumption and CO_2 emitted during the synthesis of Cu is not readily available. However, it can be estimated from the embodied energy of copper and ethanolamine since it is synthesized from the complexation these compounds. The synthesis of one more of Cu requires three moles of ethanolamine and one more of copper. Thus, the energy input during the production of Cu can be estimated as 4.34 MJ per mole Cu. The value was equivalent to 68.6 MJ per kg of Cu as Cu. Similarly, the CO_2 emission during the production of Cu was estimated as 0.492 kg CO_2-eq/mole Cu, which was equivalent to 7.74 kg CO_2-eq/ kg of Cu as Cu.

7.6. Comparison of the Proposed and Existing Technologies

7.6.1 Cu and Copper Sulfate Dose

In the previous chapters, the results from tasks that evaluated the feasibility of using Cu and copper sulfate as algal cell disruption agents were reported. The disruption of algae cells in suspension dosed with varying concentration of Cu and copper sulfate was investigated. In addition, the lipid extraction yield from algal paste dosed with varying concentration of Cu and copper sulfate was evaluated. For algal paste at 10% solids dosed with 50 and 100 mg of Cu as Cu per g of dray algae, lipid extraction yields of 190 and 210 mg/g of dry algae, respectively, were achieved at 24-hours contact time. These correspond to about 18% and 32% increases, respectively, with respect to control samples.

For copper sulfate, lipid extraction yields of 190 and 200 mg/ g of dry algae were achieved for algal paste at 10% solids dosed with 100 and 200 mg of copper sulfate as Cu per g of dry algae, respectively, at 24-hours contact time. These correspond to about 21% and 30% increases, respectively, with respect to control samples.

Cu dose of 100 mg as Cu per g of dry algae and copper sulfate dose of 200 mg as Cu per g of dry algae were used for the estimation of the energy-efficiency, cost-effectiveness, and sustainability of Cu and copper sulfate in the following subsections.

7.6.2 Energy-efficiency

In the previous subsection, it was estimated that about 68.6 MJ of energy was consumed during the production of one kg of Cu as Cu. In addition, 100 mg of Cu as Cu per g of

dry algae was determined as optimal dose. Therefore, the embedded specific energy required for disruption of algae cell with Cu was estimated as 6.86 MJ/kg dry algae.

Similarly, it was estimated that about 14.11 MJ of energy was required during the production of one kg of copper sulfate, of which over 99% was consumed during the manufacturing copper. In addition, 200 mg of copper sulfate as Cu per g of dry algae was determined as optimal dose. Therefore, the embedded specific energy required for disruption of algae cell with copper sulfate was estimated as 2.82 MJ/kg dry algae.

In Table 5, the specific energy requirement for Cu and copper sulfate, estimated in this study, and for other algal biomass disruption methods reported in the literature [16] are presented (refer to Table 1 for detailed calculations, Section 2.2). The specific energy for Cu and copper sulfate were significantly less than the requirement for hydrodynamic cavitation, 33 MJ per kg of dry algae, which was the most efficient of the existing algae cells disruption methods. The specific energy requirements for the other methods were significantly higher than that of Cu and copper sulfate; for instance, pulsed electric field required over 300 times the requirement for copper sulfate.

Moreover, the specefic energy presented in the table must be compared with the total energy avaibale in algal biomass. The total enenergy availbale by the combustion of the entire algal biomass was estimated to be about 29 MJ per kg of dry cells [16]. Except for copper sulfate and Cu, the energy requirement for the existing cell disruption methods is greater than the energy available in the biomass. It should be noted that the energy requirements presented in Table 5 don't include energy inputs for the cultivation, harvesting/ dewatering, extraction, and conversion to biofuel. Therefore, it can be concluded that the existing cell disruption methods result in a negative net energy balance.

Table 5: Specific energy requirement for selected algal cell disruption methods

Algal cells disruption methods	Energy use (MJ/ kg dry mass)
Copper sulfate (this study)	2.82
Cu (this study)	6.86
Hydrodynamic cavitation	33.00
High speed homogenizer	67.50
Sonication	132.00
Freeze drying	140.00
Microwave	420.00
Bead mills	504.00
High pressure homogenizer	529.00
Pulsed electric field	860.00

Source: The specific energy requirements for Cu and copper sulfate were estimated per the discussion presented in section 7.6.2 and refer to Table 1 in section 2.2 for other cell disruption methods.

7.6.3 Cost-effectiveness on the Basis of Operating Costs

Cu and copper sulfate are manufactured in mass and can be purchased at reasonable prices. For instance, 50 pounds (lb.) copper sulfate (25% copper) costs around $125.00 at local stores. Based on copper sulfate dose of 200 mg per g dry algae, 50 lb. of copper sulfate (12.5 lb. copper) could disrupt 28.4 kg of dry algae. The additional cost due to the use of copper sulfate was estimated at $4.40 per kg of dry algae.

Similarly, one gallon of Cu (0.909 lb. copper) costs around $36.00 at local stores. Based on Cu as Cu dose of 50 mg per g dry algae, one gallon of Cu (0.909 lb. copper) could disrupt 4.13 kg of dry algae. The additional cost due to the use of Cu was estimated at $8.73 per kg of dry algae.

Comparing the cost-effectiveness of Cu and copper sulfate with other algal biomass disruption methods is not easy. The capital cost associated with the existing disruption methods is not readily available. Therefore, operating cost was used to compare the cost-effectiveness of the proposed algal cell disruption agents with the existing methods.

For copper sulfate and Cu, the operating costs are comprised of energy use and the cost of the chemicals. The unit costs for copper sulfate and Cu were already estimated as $4.40 and $8.73 per kg of dry algae disrupted, respectively. The cost associated with energy was estimated using an average price of 12.64 cents per kilowatt-hour for electricity for industrial sector for California [47] and the specific energy use per kg of dry algae disrupted. Accordingly, the cost associated with energy use for copper sulfate was $0.10 per kg of dry algae disrupted, while it was estimated as $0.24 per kg dry algae disrupted for Cu. The total operating cost for copper sulfate was estimated as $4.50 per

kg of dry algae disrupted, while for Cu it was estimated as $9.00 per kg of dry algae disrupted.

In Table 6, the operating costs for selected algal cell disruption methods are presented. For other disruption methods, only energy cost was considered as operating cost, and it was estimated using an average price of 12.64 cents per kilowatt-hour for electricity for industrial sector for California [47] and the specific energy requirement presented in Table 5. It was determined that copper sulfate and Cu ranked at 30 and 60 percentiles, respectively, compared to existing algae cell disruption methods. On the basis of the operating cost presented in Table 6, hydrodynamic cavitation seemed to be cost-effective. However, the ranking may be changed when capital cost is taken into consideration.

Table 6: Estimated operating cost for selected algal cell disruption methods

Algal cells disruption methods	Estimated operating cost ($/kg dry mass algae)
Hydrodynamic cavitation	1.2
High speed homogenizer	2.4
Copper sulfate (this study)	4.5
Sonication	4.6
Freeze drying	4.9
Cu (this study)	9.0
Microwave	14.7
Bead mills	17.7
High pressure homogenizer	18.6
Pulsed electric field	30.2

Source: The operating costs were estimated per the discussions presented in section 7.6.3.

7.6.4 Global Warming Potential

In this project, Global Warming Potential (GWP) will be used to assess the sustainability of the algal cell disruption methods. In the previous subsections, CO_2 emission during the production of Cu was estimated as 7.74 kg CO_2-eq/kg of Cu as Cu. Based on Cu as Cu dose of 100 mg per g dry algae, the GWP for Cu as algal cell disruption agent was estimated as 0.77 kg CO_2-eq/kg of dry algae disrupted.

Similarly, during copper sulfate production about 1.3 kg CO_2-eq/kg of copper sulfate as Cu was released into the environment. Based on copper sulfate as Cu dose of 200 mg per g dry algae, the GWP for copper sulfate as algal cell disruption agent was estimated as 0.26 kg CO_2-eq/kg of dry algae disrupted.

In 2017, the California power mix included about 34% of natural gas, 29% renewables, 15% large hydropower, 9% nuclear, 4% coal, and 9% unspecified sources [48]. The lifecycle emissions for the mix was estimated as 50 g CO_2-eq./MJ of electricity generated

[49]. For hydrodynamic cavitation, the GWP was estimated as 1.6 kg CO_2-eq./kg dry algae (Table 7); higher than that of copper sulfate and Cu. Similarly, the estimated GWPs for the other disruption methods were significantly higher than that of copper sulfate and Cu, Table 7.

Table 7: Global warming potential for selected algal cell disruption methods

Algal cells disruption methods	GW (kg CO_2-eq./kg dry algae)
Copper sulfate (this study)	0.26
Cu (this study)	0.77
Hydrodynamic cavitation	1.6
High speed homogenizer	3.4
Sonication	6.6
Freeze drying	7.0
Microwave	21.0
Bead mills	25.2
High pressure homogenizer	26.4
Pulsed electric field	43.0

Source: The GWPs for the cell disruption methods were estimated per the discussions presented in section 7.6.4.

7.7. Benefits to California

Benefits to California derived from this research and development project were assessed within the following context:

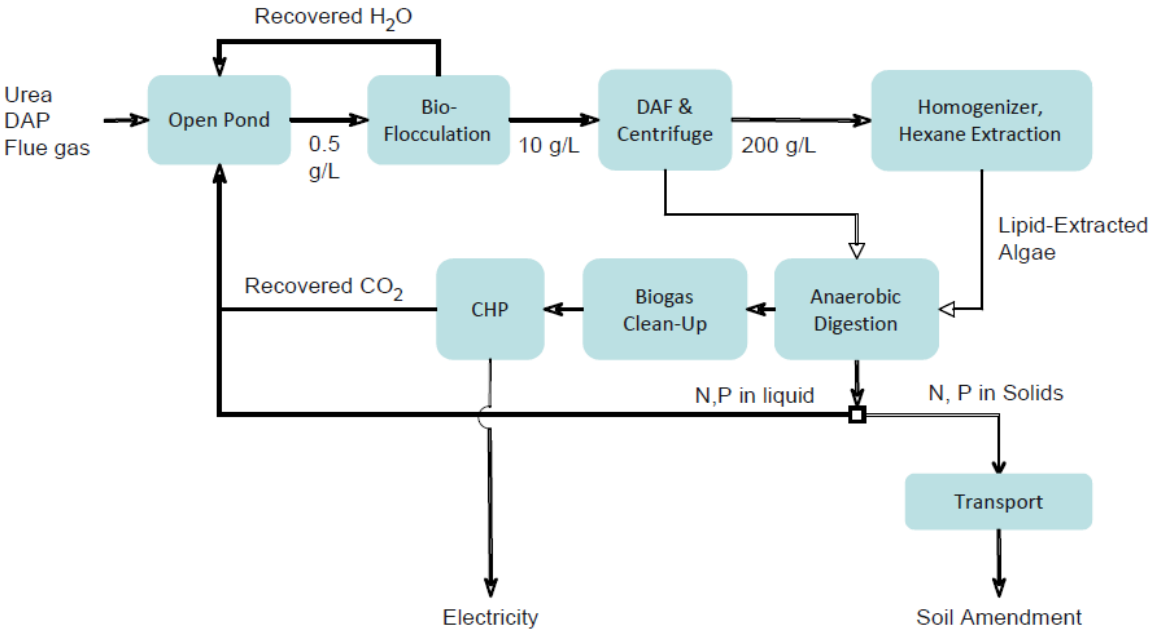
- Provide a quantified estimate of the project's GHG emissions and carbon intensity benefits.
    - Potential to help California meet its 2020 climate goal.
- Conduct TEA.
- Provide an estimate of water usage, if any, if this technology is deployed in a pilot or scale-up facility.
- Provide a quantified estimate of the project's benefits to the California biofuel industry.
    - Potential reductions in capital costs.
    - Potential reductions in operation costs.
    - Potential reductions in costs of production of algal biofuels.
- Provide a quantified estimate of the project's benefit to California.

- Potential job creation, economic development, and increased state revenue.
- Potential increase in water reuse, resource recovery from waste, and natural resources preservation.

7.7.1 Estimate of the Project's GHG Emissions and Carbon Intensity Benefits

As presented in Section 7.6.4, the GHG emissions resulting from the use of copper sulfate and Cu as algal biomass disruption agents is significantly less than the current methods. To quantify the benefit of adaption of these methods, a life-cycle analysis (LCA) for GHG emissions and energy use for algae-based biofuel performed by researchers at Argonne National Laboratory [51] was employed. For the analysis, the researchers expanded the Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation (GREET v1.6) model to include algae-based fuel production pathways. The study has included in the analysis energy recovery through biogas production from the residual biomass after lipid extraction, including fugitive methane (CH_4) emissions during the production of biogas and nitrous oxide (N_2O) emissions during the use of the solid residue from anaerobic digestion as agricultural fertilizer, Figure 18.

Figure 18: Baseline algal lipids extraction process



Source: Frank et al. 2011 [51].

The key processes used for the LCA are summarized in Table 8. The study used algae grown with CO_2 from power plant flue gas. Despite the fossil origin of this CO_2, the study treated it as atmospheric CO_2 in the analysis because power plants will operate whether algae are grown or not, and its CO_2 will be emitted into the air for the foreseeable future.

Table 8: Key processes in the Argonne's LCA for algae to biofuels pathway

Parameters	Emission Scenario
Algae growth	Open ponds mixed by paddle wheel. Water and nutrient inputs. Recovered CO_2, nutrient, and water inputs.
Harvesting/ dewatering	Dewatering with progressive steps: Bio-flocculation, dissolved air flotation, and centrifugation. Bio-flocculation allows flocculation and settling of the algae without chemical inputs.
Extraction	Algal biomass pretreatment with pressure homogenizers. Extraction with hexane is done on site proximal to the growth pond. Lipids are transported to regional centers for conversion to biofuel.
Fuel production	Extracted lipid is converted to biodiesel by transesterification.
Energy and nutrient recovery from co-products	Anaerobic digestion of the co-products for biogas production. Biogas purification. Electricity generation with combined heat and power (CHP) systems. Residual transported and used as soil amendment.

Source: Frank *et al.* 2011 [51]

For the scenario described above, the researchers estimated, using the expanded GREET Model, GHG emissions of 55.25 gCO_2-e per MJ of biodiesel produced from algal lipids. In this analysis, pressure homogenizer was employed for disrupting algae cell, and the energy requirement for the homogenizer was estimated as 183 kWh per dry metric ton (0.66 MJ per dry kg) by the researchers. This value was adapted from bio solids disintegration data and may not necessarily be applicable to algae cells. In fact, the literature data presented in Table 5 (Section 7.6.2) shows that the specific energy for algae cell disruption with high pressure homogenizer was reported as 529 MJ per kg dry algae cell. Similarly, the corresponding GHG emission was estimated as 26.4 gCO_2-e per kg of dry algae cells disrupted (Table 7, Section 7.6.4).

As presented in sections 7.6.2 and 7.6.4, the estimated specific energy requirements and GHG emissions for copper sulfate and Cu are significantly less than that of pressure homogenizer. Therefore, the carbon intensity for the adaption of Cu and copper sulfate as algal biomass disruption agent is expected to be less than 55.25 gCO2-e/MJ of biodiesel produced. This complies with the California Health & Safety, Section 44272 et seq., that the carbon intensity for diesel substitutes should be less than or equal to 83.25 gCO2-eq/MJ.

7.7.2 Potential to Help California Meet its 2020 Climate Goal

The adaption of the copper sulfate and Cu by the algal biofuel industry has a potential to help reduce California's dependence on imported fossil fuels while curbing CO_2 emissions. However, a scalable and commercially viable algal biofuel production system may not be in place by 2020 to help the state meet its climate goal. Copper sulfate and Cu as algal biomass disruption methods require further research and development and subsequent demonstration at pilot-scale, which could take up to five or more years. Moreover, the cost and energy intensity associated with the other process units along the algae-to-biofuel pathway must be reduced to make algal biofuel economically viable.

7.7.3 Techno-economic Analysis

Algal biomass has several advantages compared to other feedstock used for biofuel production. Algae have high productivity rate [5], ability to tolerate a wide range of growth conditions [6], and lack of competition for land and water with food crops [7]. Moreover, CO_2 sequestration via algae is one to two orders of magnitude higher than terrestrial plants [8]. However, the development and commercialization algal biofuel must also consider economic aspects. In fact, the cost and energy intensity of the various process units along the algae-to-biofuel pathway are currently limiting the commercial viability of algal biofuel.

A number of TEA were reported in the literature to evaluate the economic feasibility of various algae-to-biofuel pathways. In Table 9, the results from these studies were summarized.

These studies showed large variabilities in production cost – varying from $3.00/gallon to $29.80/gallon for biodiesel and from $12.80/gallon to $153.40/gallon for TAG, intimidate feedstocks for biodiesel production. The different cultivation systems, biomass productivities, oil contents, and conversion technologies employed contributed to the large variabilities in the production cost. In addition, a majority of the TEAs were performed by extrapolating laboratory-scale data, which often cannot represent the large-scale situations.

A closer look at the production costs reveal that about 60% to 75% of the overall cost was attributed to capital, while 25% to 40% to operating cost [52, 53]. Lipid extraction, which was the focus of this project, was estimated to account for about 8% of the overall capital cost for algae-to-biofuel pathways that use a pond cultivation system [53]. Electricity accounted for 7.5% and 26% of the operating cost for a pond and PBR growth systems [54], respectively. The high electricity consumption in PBR system was due to LEDs that serve as source light energy for the algae culture. In pond cultivation system, the algae culture uses solar energy.

Based on the TEA reviewed, algal biofuel is not currently competitive with petroleum-based fuels due to high production costs.

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Table 9: Techno-economic results from literature for the production of algal intermediate feedstock or biofuels

Product*	Cultivation System	Biomass Productivity	Oil Content (%)	Capacity (MM gal of product per year)	Conversion/ Upgrading Technology	Production Costs ($/gal)†	Source
TAG	Open pond	22 g/m^2/day	25	2.36	Solvent extraction	15.7	Lundquist et al. 2010 [55]
TAG	PBR	1.25 kg/m^3/day	25	10	Solvent extraction	25.5	Davis et al. 2011 [53]
TAG	Open Pond	25 g/m^2/day	25	10	Solvent extraction	12.0	Davis et al. 2011 [53]
Biodiesel	PBR	1.25 kg/m^3/day	25	9.3	Solvent extraction followed by Hydro-treating	28.9	Davis et al. 2011 [53]
Biodiesel	Open Pond	25 g/m^2/day	25	9.3	Solvent extraction followed by Hydro-treating	13.8	Davis et al. 2011 [53]
TAG	Open pond	24 g/m^2/day	50	1.9	Solvent extraction	34.7	Amer et al. 2011 [54]
Biodiesel	Open pond	24 g/m^2/day	50	1.9	Direct transesterification	19.0	Amer et al. 2011 [54]
TAG	PBR	0.9 kg/m^2/day	50	92	Solvent extraction	153.4	Amer et al. 2011 [54]

* TAG – Triacylglycerol's and must be upgraded before use as fuel source.

† All production costs have been updated to 2018 USD with the inflation rates.

Product*	Cultivation System	Biomass Productivity	Oil Content (%)	Capacity (MM gal of product per year)	Conversion/ Upgrading Technology	Production Costs ($/gal)†	Source
Biodiesel	Open pond	25 g/m^2/day	30	2.7	Solvent extraction followed by Hydro-treating	12.5	Delrue et al. 2012 [52]
Biodiesel	PBR	1.3 kg/m^3/d	30	140	Solvent extraction followed by Hydro-treating	20.8	Delrue et al. 2012 [52]
Biodiesel	Open pond	30 g/m^2/day	50	6.4	Solvent extraction followed by transesterification	3.0	Nagarajan et al. 2013 [56]
Biodiesel	Open pond	15 g/m^2/day	25	5,000	Hydrothermal liquification	9.5	Davis et al. 2014 [57]

7.7.4 Estimate of Water Usage if this Technology is Deployed in a Pilot or Scaled-Up Facility

Water usage values reported in the literature for various algae-to-biofuel pathway are summarized in Table 10. There was a large variability in the water consumption intensity reported in the literature. The type of cultivation system employed, open pond vs. PBR, significantly affects water usage. Open pond systems are prone to water loss through evaporation, while PBR limit water evaporation loss. Some algae-to-biofuel pathways recycle water, reducing water consumption per unit volume of algal biofuel production. Finally, a majority of the water usage reported in the literature were extrapolated from laboratory-scale data, which often cannot represent the large-scale situations.

Of the literature reviewed, only Delrue et al. 2012 [52] provided the water consumption breakdown for the process units along the algae-to-biofuel pathways. Accordingly, for open pond system, about 61% of the water was consumed during the cultivation step and harvesting/dewatering, extraction, and conversion each accounted for 13% of the total water usage. For the algae-to-fuel pathway that used PBR system, harvesting/ dewatering, extraction, and conversion steps each communed for 27% of the total water usage, while the cultivation process accounted for 19% of the total water consumption.

Table 10: Water usage during algal biodiesel production

Cultivation System	Water Recycle Rate (%)	Water Usage (gal water / gal of biodiesel produced)	Source
PBR	80	1,700	Subhadra et al. 2010 [58]
Open pond	0	3,400	Yang et al. 2011 [59]
Open pond	100	540	Yang et al. 2011 [59]
PBR	100	60	Harto et al. 2010 [60]
Open pond	100	650	Harto et al. 2010 [60]
Open pond	0	3,090	Delrue et al. 2012 [52]
PBR	0	1,500	Delrue et al. 2012 [52]

These reviews showed that the water consumption intensity of algal biofuel was substantial. On average, the water intensities for pond and PBR systems without water recycle were 2,650 and 3,250 gal of water per gal of biofuel, respectively. However, the water usage could be significantly reduced by water recycling. For instance, Yang et al. 2011 [59] reported about 84% reduction in water usage for a pond cultivation system with recycling. Moreover, the water consumption intensity for algal biofuel is considerably lower than the majority of feedstocks currently used or considered for biofuel production (Table 11). For examples, the water footprint for soybean biodiesel was estimated as 13,680 gallons per gallon of biodiesel produced.

Table 11: Water requirement for other type of biofuels

Biofuel Type	Feedstock	Total Water Requirement (gallon of water/ gallon of biofuel)
Bioethanol	Sugar beet	1,390
	Potato	2,400
	Sugar cane	2,520
	Maize	2,570
	Cassava	2,930
	Barley	3,730
	Rye	3,990
	Paddy rice	4,480
	Wheat	4,950
	Sorghum	9,810
Biodiesel	Soybean	13,680
	Rapeseed	14,200
	Jatropha	19,920

Source: adapted from Gerbens-Leenes et al. 2009 [61]

7.7.5 The Project's Benefits to the California Biofuel Industry

As discussed in Section 7.7.3, algal biofuel is not currently competitive with petroleum-based fuels due to high production costs. The capital cost accounted to about 60% to 75% of the overall production cost, while 25% to 40% of the total cost was attributed to operating cost [52, 53]. Electricity accounted for 7.5% and 26% of the operating cost for pond and PBR growth systems [54], respectively. Lipid extraction, which was the focus of this project, was estimated to account for about 8% of the overall capital cost for algae-to-biofuel pathways that use a pond cultivation system [53].

As presented in Section 7.6.2, the energy requirement for Cu and copper sulfate were significantly less than the requirement for current algal biomass disruption methods. Moreover, it was estimated that operating costs for copper sulfate and Cu ranked at 30 and 60 percentiles, respectively, compared to existing algae cell disruption methods; refer to Section 7.6.3. With further research and development, the adaption of the cell disruption methods developed in this project could potentially reduce the operating cost and hence the overall cost of algal biofuel production. For this benefit to be realized, further research and development is also necessary for the other process units

along the algae-to-biofuel pathway to make the overall cost of algal biomass production competitive with petroleum-based fuels.

7.7.6 Potential Job Creation, Economic Development, and Increased State Revenue

Currently, there are 100 plus companies involved in the algal biofuel arena worldwide [62], with 36 plus of them based in the US. Of the companies based in the US, 13 are located in California. Synthetic Genomics and Solazyme, based in California, are two of the top five leading algal biofuel companies globally [63]. With further research and development and subsequent pilot-scale testing, the technology developed in this project could be adapted by the algal biofuel companies based in California and elsewhere in the US.

ABO [64] estimated the potential for creation of 220,000 jobs in the algal biofuels sector by 2020 in the US. Considering the current state of the various technologies along the algae-to-biofuel pathway, the estimated job creation may not happen by 2020. However, with further research and development, the future job creation potential of the industry in California could be significant.

As stated above, of the 36 algal biofuel companies based in the US, 13 (approximately 36%) are located in California. Using ABO potential job creation data, it was estimated that about 72,000 jobs could be created in California in the future.

The biofuel industry employs a wide range of workers in a variety of occupations. Scientists and engineers conduct research and development; construction workers build plants and update infrastructure; agricultural workers grow and harvest feedstocks; plant workers process feedstocks into fuel; and sales workers sell the biofuels. According the US Bureau of Labor Statistics [65], the average annual wages for the most common occupations in the biofuel industry are presented in Table 12.

Therefore, the proposed project could benefit the state by creating green jobs. Activities related to the construction and operation of the algal biofuel plants may enhance economic development, promote economic growth, and increase revenue for the state.

Table 12: Wages for selected science and engineering occupations in biofuels

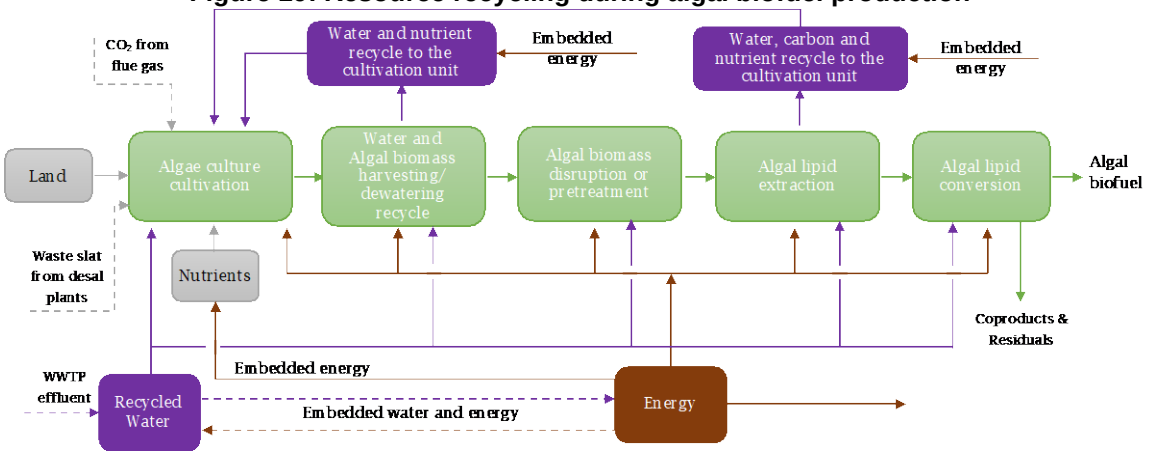
Selected Science and Engineering Occupations	Median Annual Wages, May 2011
Agricultural engineers	$74,630
Biochemists and biophysicists	$63,530
Chemical engineers	$96,870
Chemical technicians	$49,920
Chemists	$75,550
Civil engineers	$96,370
Construction managers	$101,970
Construction laborers	$29,730
Electrical engineers	$85,350
Environmental engineers	$89,070
Industrial engineers	$79,530
Mechanical engineers	$88,320
Operating engineers and other construction equipment operators	$33,440
Soil and plant scientists	$58,940

Source: Adapted from Richards, 2013 [65]

7.7.7 Potential Increase in Water Reuse, Resource Recovery from Waste, and Natural Resources Preservation

Cost and energy intensity are the two major obstacles that limited the commercialization of algal biofuel. Accordingly, further research and development is necessary for the process units along the algae-to-biofuel pathway. To address these challenges, it is recommended (see Section 8.2) that algal biofuel production could be integrated with waste treatment systems so that the resources contained in waste streams, such as effluents from WWTPs, CO_2 and thermal heat in flue gas streams, and waste salts from desalination plants, can be recovered and used as inputs to algal biofuel production systems. In addition, water, nutrient, and carbon can be recycled internally in the production of algal biofuel, Figure 19. Therefore, algal biofuel production has a potential to enhance natural resources preservation by increasing water reuse, carbon recycle, and nutrient and mineral recovery from various waste streams. This also helps in minimizing or eliminating waste release into the environment.

Figure 19: Resource recycling during algal biofuel production



7.8. Summary

In this chapter, the benefits of using Cu and copper as algal biomass disruption methods were evaluated on the basis of energy input, GHG emission, and operating cost. For agents, the estimated specific energy requirements were 5 to 300 times lower than the requirements for the existing algae cell disruption methods, and the GHG emissions were 8 to 600 times lower than those for existing methods. On the basis of operating cost, it was determined that copper sulfate and Cu ranked at 30 and 60 percentiles, respectively, compared to existing algae cell disruption methods.

Algal biofuel has an estimated GHG emissions of 55.25 gCO_2-e per MJ of biodiesel produced, which is significantly below the California Health & Safety, Section 44272 et seq., requirement of 83.25 gCO2-eq/MJ or less for diesel substitutes. The adaption of Cu and copper sulfate as algal biomass disruption methods could further reduce the GHG emissions during algal biofuel production. Algal biofuel has a potential to help the California limit its GHG emissions in the future. However, a scalable and commercially viable algal biofuel production system may not be in place by 2020 to help the state meet its climate goal.

Algal biofuel is not currently competitive with petroleum-based fuels, mainly due to cost and energy intensity. Review of TEA studies showed that cost of production varied from $3.00/gallon to $29.80/gallon for biodiesel and from $12.80/gallon to $153.40/gallon for TAG, intimidate feedstock for biodiesel production. The large variability in the cost production was attributed to different cultivation systems, biomass productivities, oil contents, and conversion technologies employed in each study reviewed. In addition, a majority of the TEAs were performed by extrapolating laboratory-scale data, which often cannot represent the large-scale situations.

About 60% to 75% of the overall cost was attributed to capital, while 25% to 40% to operating cost. Lipid extraction was estimated to account for about 8% of the overall capital cost for algae-to-biofuel pathways that use a pond cultivation system. Electricity

accounted for 7.5% and 26% of the operating cost for a pond and PBR growth systems, respectively.

The water consumption intensity of algal biofuel was substantial. On average, the water intensities for pond and PBR systems without water recycle were 2,650 and 3,250 gallons of water per gallon of biofuel, respectively. However, the water usage could be significantly reduced by water recycling. For instance, about 84% reduction in water usage for a pond cultivation system with recycling has been reported in the literature. Moreover, the water consumption intensity for algal biofuel is lower than the majority of feedstocks currently used or considered for biofuel production.

Algal biofuel is not currently competitive with petroleum-based fuels, mainly due to cost and energy intensity. It was determined that the energy requirement for Cu and copper sulfate were significantly less than the requirement for current algal biomass disruption methods. Moreover, it was estimated that operating costs for copper sulfate and Cu ranked at 30 and 60 percentiles, respectively, compared to existing algae cell disruption methods. With further research and development, the adaption of the cell disruption methods developed in this project could potentially reduce the operating cost and, hence, the overall cost of algal biofuel production. For this benefit to be realized, further research and development is also necessary for the other process units along the algae-to-biofuel pathway to make the overall cost of algal biomass production competitive with petroleum-based fuels.

ABO estimated the potential for creation of 220,000 jobs in the algal biofuels sector by 2020 in the US. Considering the current state of the various technologies along the algae-to-biofuel pathway, the estimated job creation may not happen by 2020. However, with further research and development, the future job creation potential of the industry in California could be significant.

Currently, there are 100 plus companies involved in the algal biofuel arena worldwide, with 36 plus of them based in the US. Of the companies based in the US, 13 (approximately 36%) are located in California. Using ABO potential job creation data, it was estimated that about 72,000 jobs could be created in California in the future. Therefore, the proposed project could benefit the state by creating green jobs. Moreover, activities related to the construction and operation of the algal biofuel plants may enhance economic development, promote economic growth, and increase revenue for the state.

Cost and energy intensity are the two major obstacles that limited the commercialization of algal biofuel. Accordingly, further research and development is necessary for the process units along the algae-to-biofuel pathway to make the overall cost of algal biomass production competitive compared to petroleum-based fuels. It is recommended (see Section 8.2) that algal biofuel production could be integrated with waste treatment systems so that the resources contained in waste streams, such as effluents from WWTPs, CO_2 and thermal heat in flue gas streams, and waste salts from

desalination plants, can be recovered and used as inputs to algal biofuel production systems. Therefore, algal biofuel production has a potential to enhance natural resources preservation by increasing water reuse, carbon recycle, and nutrient and mineral recovery from various waste streams. This also helps in minimizing or eliminating waste release into the environment.

8. CHAPTER 7: Conclusions and Recommendations

8.1. Conclusions

The project showed that Cu and copper sulfate were effective in disrupting algae cells. Cellometer and SEM analyses of the samples confirmed that the algae cells were ruptured after treatment with the agents. The rupturing effect has increased lipid extraction yield by 32% with respect to control samples for 10% paste dosed with 100 mg of Cu as Cu per g of dry *C. vulgaris* and 24-hours of contact time. About 30% increase in lipid yield was achieved for copper sulfate at 200 mg of Cu per g of dry algae. On the basis of the mass of copper dosed, it was also observed that copper sulfate was less effective compared to Cu.

It was also observed that a contact time of 24-hour was needed to achieve significant increase in lipid yield, and this may limit the adaption of Cu and copper sulfate as algal cell disruption methods. This is because longer contact times require large reactor volumes, which may in turn increase the cost of the technology.

A GHG emissions potential of 55.25 gCO_2-e per MJ was estimated for biodiesel produced from algal lipids. With further research and development, the adaption of Cu and copper sulfate as algal biomass disruption method has a potential to reduce the GHG emissions further below the California Health & Safety, Section 44272 et seq., requirement of 83.25 gCO2-eq/MJ or less for diesel substitute.

The water consumption intensity of algal biofuel was substantial. On average, the water intensities for pond and PBR systems without water recycle were 2,650 and 3,250 gallon of water per gallon of biofuel, respectively. The water usage could be significantly reduced by water recycling. About 84% reduction in water usage has been reported for a pond cultivation system with recycling. Moreover, the water consumption intensity for algal biofuel is considerably lower than the majority of feedstocks currently used or considered for biofuel production.

Biofuel production from algal biomass has a potential to enhance natural resources preservation by increasing water reuse, carbon recycle, and nutrient and mineral recovery from various waste streams. This also helps in minimizing or eliminating waste release into the environment.

Despite these advantages, algal biofuel is not currently competitive with petroleum-based fuels, mainly related cost and energy intensity. The cost of production varied from $3.00/gallon to $29.80/gallon for biodiesel and from $12.80/gallon to $153.40/gallon for TAG, intimidate feedstock for biodiesel production. About 60% to 75% of the overall cost was attributed to capital costs, while 25% to 40% to operating

costs. Electricity accounted for 7.5% and 26% of the operating cost for a pond and PBR growth systems, respectively.

While the project results point out that algal biofuel production is not competitive with fossil technology, it also appears that algal solutions for the transportation sector requires significant investments, technological development, and time. It is not clear that the private sector is willing to make the requisite investments needed or that the public sector has the capacity to invest in this technology for the long term in reducing technological costs. Equally evident is the absence of when the public may see commercial algal products in the transportation sector.

8.2. Recommendations

Future research and development efforts must be focused on reducing the contact time between the disruption agents and algae cell. This may be achieved by using copper nanoparticles, where the surface area to volume ratio is very large. The larger surface area will increase the reactivity of copper with algae cell, thereby reducing the contact time.

It is also recommended that the technology must be pilot-tested to identify and address challenges that may arise during upscaling. The data that will be gathered from pilot-scale test would be used to establish the process and operational parameters for the technology, paving the way for eventual commercialization.

Moreover, further research and development is necessary for the other process units along the algae-to-biofuel pathway to make the overall cost of algal biomass production competitive compared to petroleum-based fuels. Cost and energy intensity are the two major obstacles that must be overcame. To address this, it is recommended that algal biofuel production could be integrated with waste treatment systems so that the resources contained in waste streams can be recovered and used as inputs to the algal biofuel system. Effluents from WWTPs could serve as source of water, nutrient supplement, and carbon source. Flue gas streams from power plants and other industries provide CO_2 and thermal energy, in the form of low-grade heat. Finally, waste salts from desalination plants can be used as mineral sources for algae culture cultivation. Besides the availability and characteristics of these resources, their geo-location with respect to the siting of the integrated system is critical. Therefore, performing comprehensive analyses of the resources, the siting of the integrated system, and the logistics in the transportation of some of the resources is warranted. Finally, TEA of the integrated system is vital to determine the economic feasibility of algal biofuel production with the integrated system.

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10. Glossary

AB – Assembly Bill

ABO – Algal Biomass Organization

AMDIS – Automated Mass Spectral Deconvolution and Identification System

C. vulgaris – *Chlorella vulgaris*

DI – Deionized

FAME – Fatty Acid Methyl Ester

G – Gravitational Constant

GC – Gas Chromatograph

GHG – Greenhouse Gas

GREET – Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation

GWP – Global Warming Potential

J – Joule

k – Intrinsic Rate Constant

k_{sp} – Pseudo Rate Constant

LED – Light-Emitting Diode

LCA – Life-Cycle Analysis

MJ – Mega joule

MSD – Mass Selective Detector

NIST – National Institute of Standard and Technology

PBR – photo bioreactor

PI – Propidium Iodide

pJ – Pico joule

PTFE – Polytetrafluoroethylene

SEM – Scanning Electron Microscope

TEA – Techno-economic Analysis

TAG – Triacylglycerol's

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